A LABORATORY MANUAL

OF

Physiological Chemistry

BY

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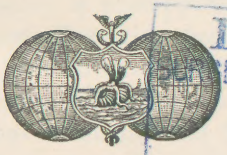
UNIVERSITY OF IOWA

WITH ONE COLORED PLATE AND THREE PLATES
OF MICROSCOPIC PREPARATIONS.



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PREFACE.

In view of the results attained from the course given in physiological chemistry in this University, as well as the experience of others, the author is firmly convinced of the superiority of the laboratory method of instruction over the didactic, believing that it is only by practical work that the student can become familiar with the physiological changes in progress in the animal body and their products. This book has been prepared with the aim of imparting accurate knowledge through the student's own observation. It has seemed advisable to include with the directions for experimental work a brief explanation of the facts observed, so as to call attention to their meaning; or, at times, to state others which are important, but which could not well be demonstrated in such a course as this. Some acquaintance with general chemistry and with chemical manipulation is presuppósed.

For the purpose of making the course flexible, the less important experiments, or those which are not of general interest, have been printed in smaller type. A few blank pages have been inserted for additional notes by the student. It has been found that the time usually assigned to chemistry in one year of a medical course is sufficient for the performance of most of the experimental work.

As far as possible, the work has been so arranged as to require but a small stock of apparatus and reagents

and such as are readily obtainable. By this means a large class can carry on the work together. Complicated experiments have been omitted or put in small type for the use of advanced students or those who choose to spend more time upon the subject.

The animal substances which are required—albumin, blood, bile, and others—can be found in the market or obtained from the slaughter-house. If no hospital is near, gastric juice, urine, etc., corresponding to pathological specimens can be prepared artificially for testing by the student. The expense of the course is very small.

ELBERT W. ROCKWOOD.

UNIVERSITY OF IOWA,
JULY 31, 1899.

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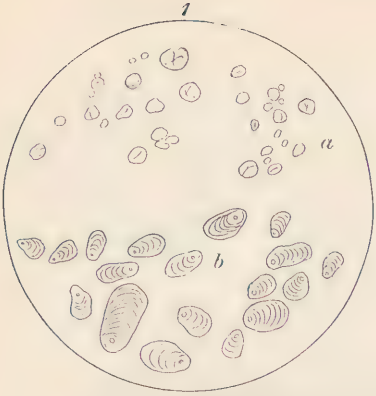
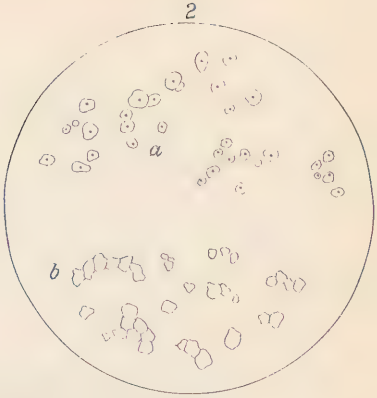
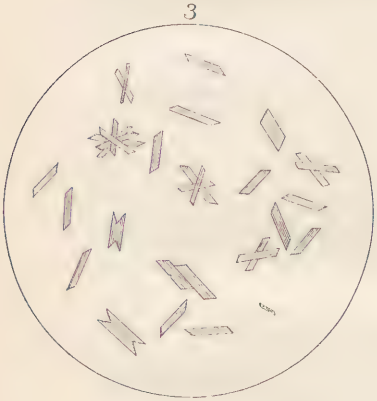
PLATE I.

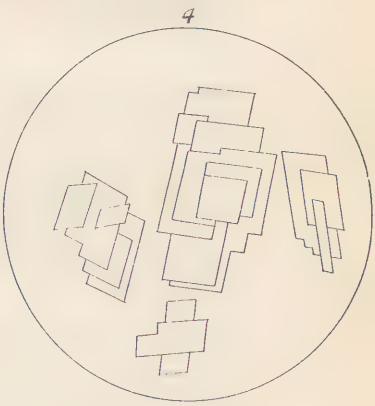
1. *a*, Wheat-starch granules.
   b, Potato-starch granules.
2. *a*, Corn-starch granules.
   b, Buckwheat-starch granules.
3. Hæmin crystals, color brown.
4. Cholesterin, colorless, transparent.
5. Phenyl-glucosazone, yellow.
6. *a*, Urea, colorless.
   b, Urea nitrate, colorless.

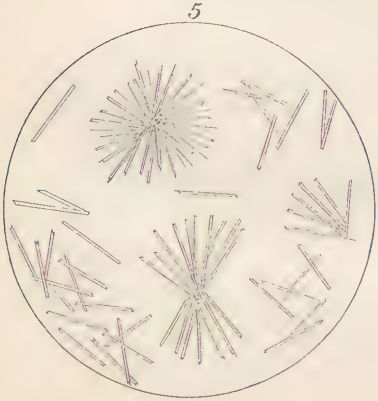
PLATE I.











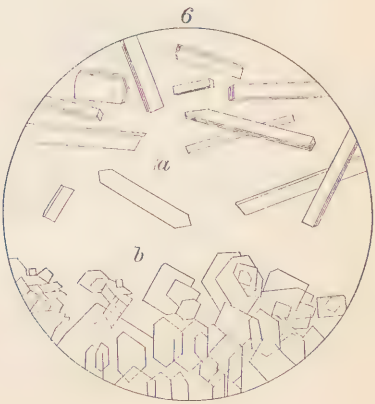


PLATE II.

7. Calcium phosphate, crystallized and amorphous forms, both colorless.
8. Triple phosphate, "coffin-lid" crystals, colorless.
   Sodium urate, brown spherical masses with spicules.
   Bacteria.
9. Ammonium urate, "thorn-apple" forms, color brown.
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11. Uric acid crystals, yellow to dark brown.
    Amorphous urates, brownish.
12. *a*, Calcium sulphate crystals, colorless.
    b, Impure leucin, nearly colorless.
    c, Tyrosin, colorless when pure.

PLATE II.

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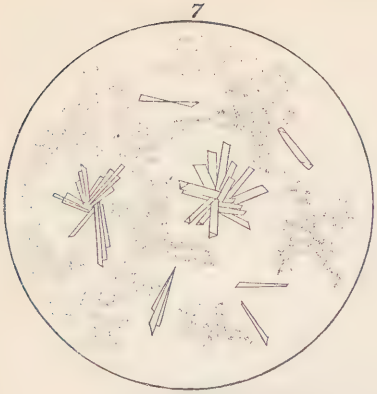

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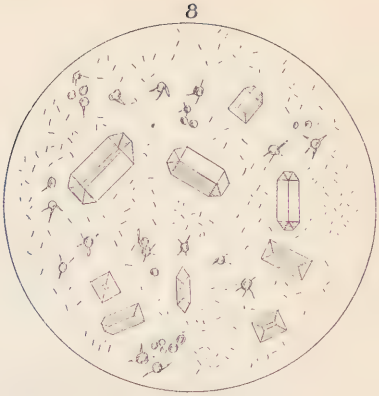

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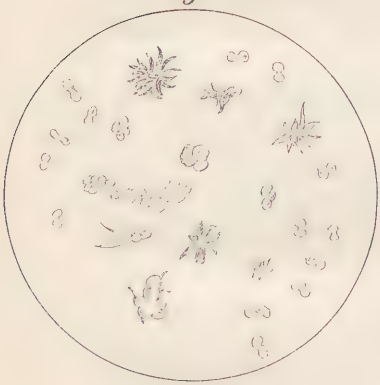

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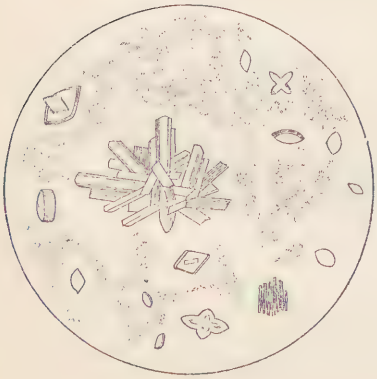

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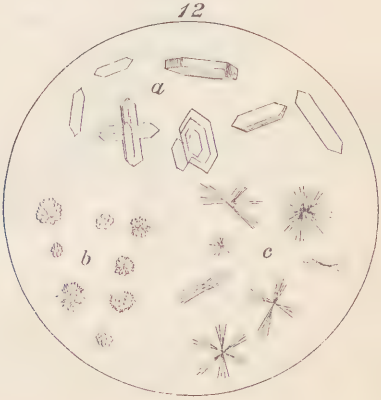

PLATE III.

13. *a*, Normal pus-corpuscles or mucus-corpuscles, granular.
    b, Pus-corpuscles swollen with acetic acid, showing nuclei.
    c, Pus-corpuscles showing amœboid movement. All colorless.
    d, Blood-corpuscles, nearly colorless.
14. Different forms of epithelial cells, colorless.
15. Granular casts, colorless.
16. Epithelial casts, colorless.
17. Hyaline casts.
    a, Broad or waxy, colorless.
    b, Narrow, colorless, and extremely transparent.
18. *a*, Fat-casts.
    b, Yeast-fungi in urine.
    c, Spermatozoa.

PLATE III.

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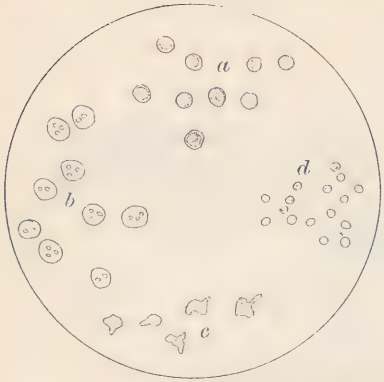

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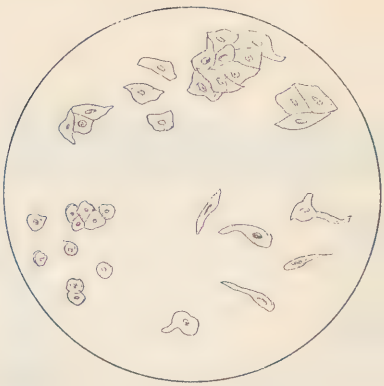

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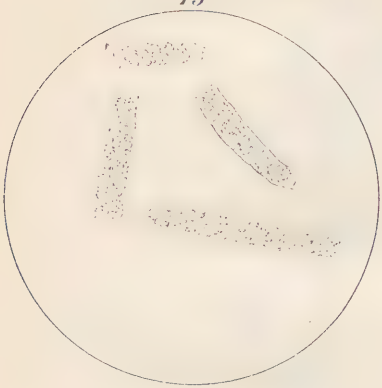

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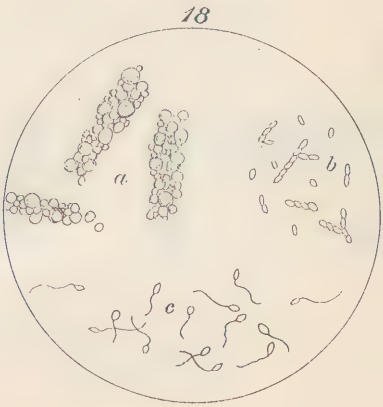
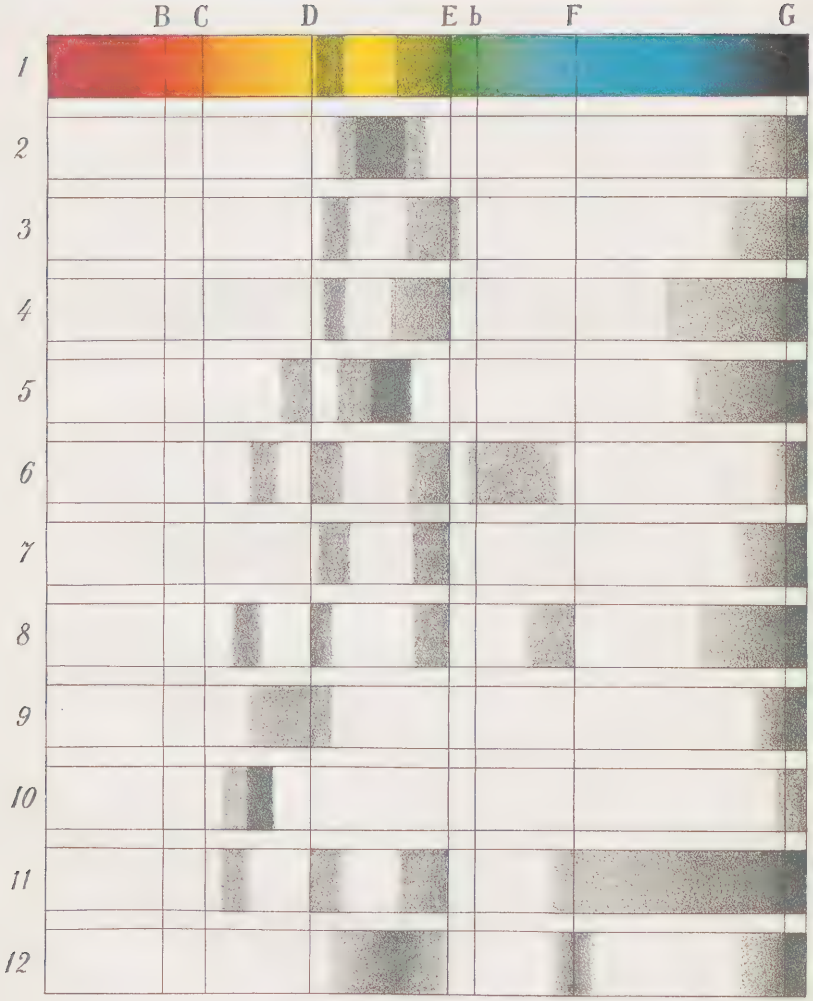


PLATE IV.

ABSORPTION-SPECTRA.

1. Oxyhæmoglobin.
2. Hæmoglobin.
3. CO-hæmoglobin and CO-hæmochromogen.
4. Methæmoglobin, alkaline.
5. Hæmatoporphyrin, acid.
6. Hæmatoporphyrin, alkaline.
7. Hæmochromogen, alkaline.
8. Hæmatin, acid.
9. Hæmatin, alkaline.
10. Sulphur methæmoglobin.
11. Methæmoglobin, neutral or faintly acid.
12. Pettenkofer's test for biliary acids.

PLATE IV.



INTRODUCTION.

The principal materials which enter into the composition of the animal body, as well as of the food necessary for its support, may be divided into several general classes:—

I. Inorganic { 1. Water. 2. Mineral substances.

II. Organic { 1. Non-nitrogenous compounds, such as { (*a*) Carbohydrates. (*b*) Fats. 2. Nitrogenous compounds, such as the Proteins.

There are a number of nitrogenous compounds in the animal body which cannot be classed under the proteins, and others which contain no nitrogen, but which do not belong to the carbohydrates or fats; nevertheless, these three classes include, by far, the largest part of the organic constituents.

THE CARBOHYDRATES.

The carbohydrates are composed of three elements: carbon, hydrogen, and oxygen. The latter two are always present in proportion to form water, and in this the carbohydrates differ from the fats, which contain less oxygen. The name of the group is derived from their composition,

although they cannot be made directly from carbon and water. Most of them contain in a molecule six atoms of carbon or some multiple of six. Many organic compounds of carbon, hydrogen, and oxygen, however, have the two latter in the proportion to form water, but do not belong to the carbohydrates.

They are found in both the animal and vegetable kingdoms, but are most abundant in the latter.

The different members of the group differ greatly in their properties, such as the power of crystallization, fermentation, reducing effect, action on polarized light, taste, etc.

They may be divided according to their molecular composition into three classes:—

I. Glucoses, or monosaccharids, $C_6H_{12}O_6$, including:
   1. Glucose, or grape-sugar; also called dextrose.
   2. Fructose, or fruit-sugar; also called lævulose.
   3. Less important are galactose, mannose, and several others.

II. Saccharoses, or disaccharids, $C_{12}H_{22}O_{11}$, including:—
   1. Sucrose, or cane-sugar.
   2. Lactose, or milk-sugar.
   3. Maltose, or malt-sugar, and some others.

III. Amyloses, or polysaccharids, $(C_6H_{10}O_5)_x$, including:—
   1. Starch.
   2. Dextrin.
   3. Glycogen.
   4. Cellulose; also a number of gums and others of less importance.

In addition to the above classes there are a number of compounds containing three, four, five, seven, eight, or nine atoms of carbon in a molecule. As yet they are not known to be of any great importance in physiological chemistry.

The first class, the monosaccharids, is so named because they contain one of the groups of six carbon atoms. They are mostly crystalline, easily soluble in water, and have a sweet taste. Chemically they are aldehyde or ketone compounds of the hexatomic alcohols. They have the power of reducing the oxygen compounds of the metals and of forming compounds with phenyl-hydrazin. They will also undergo fermentation with yeast.

The disaccharids contain in a molecule twelve atoms of carbon. They may be conceived of as composed of two molecules of a monosaccharid minus one molecule of water:

$$C_6H_{12}O_6 + C_6H_{12}O_6 = C_{12}H_{22}O_{11} + H_2O.$$

By the action of acids or ferments they take up a molecule of water and form two molecules of a monosaccharid. This formation of one or more simple sugars from a molecule of a disaccharid is called inversion, and the resulting sugar is known as invert-sugar. The disaccharids do not undergo fermentation with yeast until they have been inverted. They are all soluble in water and have a sweet taste.

The polysaccharids contain more than two groups of six carbon atoms, though the number is, in most cases, not positively known, and therefore is represented by x. They have probably a much higher molecular weight than the other classes. Their constitution is not known. They are mostly amorphous, insoluble in water, and, consequently,

tasteless. Ferments and acids convert them into the monosaccharids. They do not have reducing power.

Starch.

Starch occurs in the cells of the plant. It is in the form of grains or granules, which vary in size in different plants from about 0.002 millimeter to ten times that. The granules are composed of two parts: an inner, soluble one, called granulose, and an outer one, called cellulose. This latter is insoluble in water and protects the starch from the action of many of the weaker ferments. When boiled or acted upon by alkalies it is broken, allowing the granulose to escape and forming starch-paste, or soluble starch. The shape and size of the granules differ so much in the different plants that the source can often be determined by its microscopic appearance. Those of the potato have a shape somewhat similar to that of a clam-shell, those of wheat are round and smaller, and those of buckwheat more irregular. (Plate I, 1 and 2.)

Starch can be obtained from the parts of the plant where it is stored up, like the tuber of the potato or the kernel of grain, by macerating it, then washing out the starch with cold water.

Starch is a colloid. A colloid is a substance which when dissolved will not pass through an animal membrane or parchment. They are the opposite of crystalloids, which are usually crystalline and which will diffuse through such membranes. This process of diffusion or separation of colloid from crystalloid substances is called dialysis. As starch cannot pass through an animal membrane, it must be changed to a diffusible form before it can be absorbed. This is effected by ferments in the saliva and pancreatic fluid.

By heating to 160° to 200° starch is converted into dextrin.[1] By boiling a solution with a dilute acid it is changed first into dextrin, then into glucose. Ptyalin changes it first into dextrin, finally to maltose. The diastase of malt gives the same products.

Starch gives an intense-blue color with a solution of iodin. This color disappears on heating the liquid; but if it is not heated too long it becomes blue or purple again when it cools. The color will also be destroyed by the addition of anything which will form a compound with the iodin, such as sodium thiosulphate, silver salts, or the alkaline hydrates.

EXPERIMENT 1.—Starch may be prepared from a potato by grating it upon a tin grater, stirring it up with a little water, and squeezing the water, which contains a large part of the starch, through a piece of unbleached muslin. After repeating this with several portions collect the water in one vessel and allow the starch to settle to the bottom. Pour off the water, add more, and allow to settle again, repeating till the starch appears clean and white. Take what is needed for the experiments and let the rest dry. Enough starch for the microscopic examination can be obtained from the scrapings from a potato without washing. The cellulose-fibers will then be seen also.

EXPERIMENT 2.—Examine the starch under the microscope. Notice the shape of the granules.

EXPERIMENT 3. — Place a drop of very dilute iodin solution upon the slide so that it runs under the cover-glass and notice the markings which are thus brought out upon the granules which are least colored.

EXPERIMENT 4.—Examine in the same way starch

[1] Unless otherwise stated, all degrees of temperature will be understood as referring to the centigrade scale.

from other sources: corn, wheat, buckwheat, etc. Observe the difference in the size and shape of the granules.

EXPERIMENT 5.—Prove that starch does not dissolve in cold water by filtering after shaking powdered starch in a test-tube of water. Iodin gives no color to the filtrate.

EXPERIMENT 6.—Add about a gramme of starch to 100 cubic centimeters of cold water, mix it thoroughly, and boil. The starch has dissolved, as is shown by filtering and, after cooling, testing a portion of the filtrate with iodin solution. A deep-blue color is produced. It is destroyed by heating, but reappears as purple or blue again upon cooling.

EXPERIMENT 7.—Tie a piece of animal or vegetable parchment over the end of a funnel, or use instead a piece of parchment dialyzing tube. First see that this does not leak. It should hold water when suspended by the two ends. Place inside some of the starch solution made in the preceding experiment and hang the whole in a small beaker of water, so that the liquids inside and outside are at the same level. Allow it to stand several hours, then test the water outside with iodin for starch. It does not pass through because it is a colloid.

EXPERIMENT 8.—Examine under the microscope the starch-paste which has been made by heating starch in water. The granules have been burst open and destroyed.

Prove that starch can be decomposed by acids or ferments by means of the following:—

EXPERIMENT 9.—In about 100 cubic centimeters of water in a porcelain dish boil enough starch, previously moistened with cold water, to make a thin paste. Add about 10 cubic centimeters of dilute H_2SO_4 and boil, stirring at first, until the liquid becomes thinner. Keep the solution up to its original volume by the addition of water.

If this is not done the strong acid will turn the liquid brown or black. From time to time remove a portion, cool, and test with iodin. When the iodin gives a red color the starch has been converted into dextrin. When no color appears on the addition of iodin it has been changed to glucose. Test a portion for glucose by adding an equal volume of sodium hydrate solution, then, drop by drop, cupric sulphate solution till a deep-blue color is produced. Heating this will give a yellow or red precipitate, showing the presence of glucose. This is known as Trommer's test for glucose.

EXPERIMENT 10.—Try Trommer's test with the starch solution. It does not respond.

EXPERIMENT 11.—Add gradually to the remainder of the solution which has been boiled with the acid, while it is still hot, powdered calcium or barium carbonate until it is neutral. Filter and evaporate the filtrate on the steam-bath.[1]

DEXTRIN.

Dextrin is the intermediate product in the change from starch to glucose or maltose. There have been several varieties described: erythrodextrin, which is colored red by iodin; achroödextrin, which is not so colored, etc.

It is formed from starch by the action of heat, acids, or ferments. It is soluble in water, making a sticky liquid, often used for a mucilage. It is produced when bread is toasted, and is also found in the crust. Toast or bread-

[1] To evaporate a liquid on a steam- or water- bath the evaporating dish in which it is contained can be heated by standing it on a beaker of boiling water. This removes all danger of burning the residue.

crust, then, has its starch partially changed into a diffusible substance.

EXPERIMENT 12.—Prepare dextrin from starch by heating in a porcelain dish on a sand-bath half a spoonful of powdered starch previously dampened with a few drops of dilute nitric acid made by adding a few drops of nitric acid to a test-tube-ful of water. The starch must be stirred with a glass rod until it has turned yellowish or brown, when it has been changed to dextrin.

EXPERIMENT 13.—Dissolve some dextrin in water and test with a drop of iodin solution. A red or brown color is produced, not a blue, if the change has been complete. If commercial dextrin is tested it will probably be found to contain undecomposed starch.

GLYCOGEN.

Glycogen is found in a few of the lower plants, in some shell-fish, and in many fluids and tissues of the bodies of mammals. It is most abundant in the liver, and next in the muscles. It is also called liver-sugar or liver-starch. In the animal body it is most plentiful when the animal is well nourished, especially after a full meal. At such times it may be in as large an amount as 10 or 12 per cent. of the liver, but it is usually not more than 3 or 4 per cent. It disappears completely from the liver after long starvation, or more quickly through severe work or great fright.

It is best obtained from the liver. After boiling to kill the ferments which are always present, dissolving in water, and removing the nitrogenous substances, it can be precipitated by alcohol.

Glycogen is an amorphous, white, tasteless powder. In water it dissolves to an opalescent solution. With iodin it gives a red color, which disappears on heating. It does

not have a reducing action upon cupric hydrate. Boiling with acids converts it into dextrin, then maltose, then glucose. The salivary and pancreatic ferments produce the same change.

The glycogen of the liver seems to be formed mostly from the carbohydrates of the food, but partly, at least, from the nitrogenous compounds.

It is deposited in the liver as a reserve material, just as the starch is stored for a reserve material in the plants. When it is needed by the body it is converted by a ferment into grape-sugar, and this passes into the circulation. It is probable that it is used to furnish energy for the body. After death the glycogen quickly disappears from the tissues of the body, being decomposed by the ferments which are present. If these are destroyed by boiling the tissue for a short time the glycogen is not destroyed, but can be extracted.

Experiment 14.—Prepare glycogen from the liver of a freshly-killed, well-nourished animal. The animal is best killed while digestion is in progress. Remove the liver as soon as possible, cut it into lumps, and immediately put it into about four times its weight of boiling water. Let it boil half an hour, then rub up the lumps as much as possible in a large mortar, add water, and boil again. Filter through muslin, concentrate upon the water-bath to about one-fourth its volume, and allow the solution to cool. Then precipitate the gelatin and other protein compounds by adding alternately small quantities of hydrochloric acid and potassio-mercuric iodid[1] as long as anything is thrown

[1] Prepare by precipitating mercuric chlorid with potassium iodid, washing the precipitate and then adding it to a hot solution of potassium iodid as long as it dissolves.

down. Filter and add to the filtrate twice its volume of alcohol to precipitate the glycogen. Wash with alcohol. To purify the substance it should be dissolved in a little water and precipitated again with alcohol. If the anhydrous powder is desired, the water must be removed as far as possible before drying. To accomplish this wash the precipitate next with absolute alcohol, then with ether to remove the alcohol. Dry in a vacuum-desiccator over sulphuric acid. If the pure substance is not desired, the tests may be made on the solution after the removal of the protein compounds.

EXPERIMENT 15.—If the dry substance has been obtained, try its taste and its solubility in water. Test the solution with iodin. It gives a red color.

EXPERIMENT 16.—Try Trommer's test. There is no red color if the glycogen has been purified. If it has not been it contains glucose, which responds to the test.

EXPERIMENT 17.—Convert one portion of the solution into glucose by heating with HCl and another by the action of saliva. Test for the glucose by Trommer's test.

EXPERIMENT 18.—Prove that the glycogen is destroyed (changed to glucose) in the liver after death by the action of a ferment, making the test upon some liver from the market. Chop it finely and extract with boiling water. Acidify the solution slightly with acetic acid, add a little sodium chlorid, and boil to precipitate the protein compounds. After filtering, test the filtrate for glycogen by means of iodin and also for glucose by Trommer's test.

EXPERIMENT 19.—Add a little blood to a test-tube of the glycogen solution and after it has stood ten minutes in a beaker of water at body-temperature slightly acidify with acetic acid, boil, and filter to remove the albumin, and test the filtrate for glucose and glycogen. The latter has

been converted into glucose by a ferment which is found in the blood.

Cellulose.

Cellulose forms the membrane of the plant-cells, and is not found as a constituent of the animal body, except in a few of the lower forms. Cotton and filter-paper are two of the most common examples. It is distinguished from the other polysaccharids by its insolubility. It is insoluble in the ordinary solvents, but can be dissolved in the strong mineral acids, being converted into dextrin. It also dissolves in a solution of cupric hydrate in ammonia. (Schweitzer's reagent), and in a solution of zinc chlorid (Schultze's reagent). Sulphuric acid changes paper into a parchment-like substance by covering the surface with a coating of its decomposition-products and so sticking the fibers together. Iodin does not stain the unaltered cellulose, but does so after it has been acted upon by the acid. Cellulose is only slightly attacked by the digestive ferments of man, though the herbivorous animals digest it to a greater extent. By the continued action of acids it is converted into glucose.

Experiment 20.—Show that cellulose is not stained by iodin.

Experiment 21.—Try the solubility of cotton or filter-paper in solution of zinc chlorid (Schultze's reagent) and also in a solution of cupric hydrate in ammonium hydrate (Schweitzer's reagent). It can be precipitated from these solutions by dilution with water.

Experiment 22.—To one volume of water in a beaker add slowly two volumes of concentrated sulphuric acid, stirring meanwhile. Cool the mixture; then immerse in it for a few seconds a piece of heavy filter-paper, plunging

it into a large beaker of cold water as soon as it is removed. If the time of immersion has been correct it will be semi-transparent after washing, and as tough as an animal membrane. It is called vegetable parchment. It can be stained blue by iodin.

Experiment 23.—Let another piece of paper remain in a small amount of the warm acid until it has entirely disappeared. Then dilute a little of the acid with water and test it for glucose, being sure that enough alkali has been added to give it an alkaline reaction.

Glucose ($C_6H_{12}O_6$).

Glucose is also called dextrose and grape-sugar. It is found in the vegetable kingdom as well as in the animal. It is normally present in the blood and lymph and in other fluids of the body. Pathologically it is found in considerable quantities in the urine, sometimes in as large amounts as 10 per cent. or more. The urine may also temporarily contain grape-sugar after a diet rich in carbohydrates. Whether it may normally occur in very small amounts in the urine is a question which is often discussed, but upon which there is no general agreement.

Glucose is made commercially by boiling starch with a dilute acid. It can be produced from any of the polysaccharids or disaccharids in the same manner. They unite with one or more molecules of water, forming glucose:—

$$(C_6H_{10}O_5)_x + x(H_2O) = x(C_6H_{12}O_6).$$
$$C_{12}H_{22}O_{11} + H_2O = 2C_6H_{12}O_6.$$

Pure glucose can be made from pure cane-sugar by dissolving it in alcohol and adding hydrochloric acid. The glucose crystallizes out on standing.

Glucose is a crystalline substance, but crystallizes with difficulty from water. It can better be crystallized from methyl alcohol or ethyl alcohol. Its taste is sweet, but less so than that of cane-sugar. It is easily soluble in water or hot alcohol. With yeast, glucose ferments best at about 25° C., forming alcohol and carbon dioxid:—

$$C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2.$$

In the presence of milk or cheese it ferments to lactic acid. Calcium carbonate or oxid of zinc must be added to keep the solution neutral if it is desired that the action go on for a long time, as the presence of the acid kills the ferment:—

$$C_6H_{12}O_6 = 2C_3H_6O_3.$$

By the action of another ferment the lactic acid is changed into butyric acid:—

$$2C_3H_6O_3 = C_4H_8O_2 + 2CO_2 + 4H.$$

Experiment 24.—Prepare pure glucose from cane-sugar by the following method:—

Acidify 100 cubic centimeters of 90-per-cent. alcohol with 4 cubic centimeters of concentrated hydrochloric acid, warm the liquid upon the water-bath to 45°, and add gradually 30 grammes of finely-powdered cane-sugar, stirring until it has dissolved. The temperature should not rise above 50°. After two hours at 50° the sucrose has been inverted. Then let it stand in a cool place. The glucose commences to crystallize out in about a week, but crystallization may be hastened by adding to the cold solution a few crystals of glucose and by frequent stirring. After the glucose has crystallized from the solution filter, best with the aid of a filter-pump; wash free from the acid by 90-per-cent. alcohol, then by absolute alcohol; finally dry the crystals. It may be purified by dissolving in pure methyl alcohol by the aid of heat and allowing it to again crystallize out.

EXPERIMENT 25.—Prove that cupric hydrate (made by the addition of a few drops of copper sulphate to a sodium hydrate solution) is soluble in a solution of glucose, giving a deep-blue liquid. Also show that this blue solution of copper and sugar is decomposed by heating, and yellow or red precipitate of cuprous oxid is produced. This is Trommer's test for grape-sugar.

EXPERIMENT 26.—Show that cupric hydrate is also soluble in a solution of Rochelle salt or glycerin in water, but that these solutions are not decomposed by boiling. Add to each of them a little grape-sugar and heat. Cuprous oxid is formed in both cases. The former is called Fehling's test, the latter Haines's test for glucose.

EXPERIMENT 27.—Show that a glucose solution will reduce copper acetate acidified with acetic acid[1] when heated for some time in a water-bath. This is Barfoed's test. Observe the difference between glucose and lactose with this test.

EXPERIMENT 28.—Prove that when heated alone in water cupric hydrate gives the black cupric oxid and not the red cuprous oxid; also that with an excess of the copper solution the black may hide the red oxid if only a small amount of sugar is present.

EXPERIMENT 29.—Show that glucose will also reduce the subnitrate or basic nitrate of bismuth if the solution is made alkaline by sodium hydrate or carbonate and boiled. The bismuth oxid which is formed is a black powder, but if mixed with much of the unreduced bismuth subnitrate it may appear gray. This is Boettger's test. The bismuth subnitrate, like cupric hydrate, is soluble in an alkaline

[1] Reagent must contain 0.5 per cent. of acetic acid and from 0.5 per cent. to 4.0 per cent. of copper acetate.

solution of Rochelle salt, and this solution when heated with glucose gives the black oxid as a precipitate (Nylander's test).

Heat a little of the bismuth subnitrate in a solution of albumin which has been made strongly alkaline with sodium hydrate, and notice that the sulphid of bismuth, which is formed, has the same appearance as the black oxid which is produced by the glucose; that is, albumin gives a similar result to that obtained with grape-sugar.

EXPERIMENT 30.—Dissolve in a small amount of water as much phenyl-hydrazin hydrochlorid as can be taken up on the point of a knife and twice as much sodium acetate, filtering if it is not clear. Add it to half a test-tubeful of the sugar solution, place the tube in a beaker of boiling water, and heat it for an hour. Then cool it and examine the precipitate with the microscope. It is phenyl-glucosazon: bright-yellow, needle-shaped crystals. They may be single, but are more often in clusters (Plate I, 5). They can be distinguished, if necessary, from similar compounds of the other sugars by their melting-point, which is 204° C. If they separate in the amorphous state, they may be crystallized, after filtering, by dissolving in a little hot alcohol, then evaporating the alcohol to a small volume, and letting it stand.

EXPERIMENT 31.—Crush a piece of condensed yeast as large as a pea in a test-tube of water, and wash it two or three times by decantation to remove any fermentable substances which may be present. Fill the tube completely full of a glucose solution. Mix and place it, still full of the liquid, with the mouth downward in a beaker which contains a little water, or, better, some of the grape-sugar solution. Let it stand for twenty-four hours in a warm place. The carbon dioxid, which is formed, is found in the test-

tube and the alcohol in the liquid. The gas may be proved to be carbon dioxid by shaking it with lime-water, which it turns white. The presence of the alcohol is shown by warming the liquid after the addition of sodium hydrate and a little iodin. Iodoform separates out in yellow scales, or, if the amount of alcohol is very small, the odor alone may be perceived.

QUANTITATIVE TEST FOR GLUCOSE.

Fehling's Method.—The solutions used are: (*A*) 34.64 grammes of cupric sulphate ($CuSO_4$, $5H_2O$), dissolved in enough water to make the volume 500 cubic centimeters. The crystals used must be dark blue and not effloresced; (*B*) 187 grammes of pure Rochelle salt and 68 grammes of sodium hydrate in water enough to make the volume 500 cubic centimeters. These solutions must be kept separate.

EXPERIMENT 32.—For each determination mix 5 cubic centimeters of *A* with 5 cubic centimeters of *B*, measuring carefully with a pipette. Add about 40 cubic centimeters of water, and heat to boiling in a beaker or porcelain dish. If the solution is good there will be no red precipitate.

The best results are obtained when the solution contains from 0.5 per cent. to 1.0 per cent. of sugar; that is, when from 5 to 10 cubic centimeters are necessary to destroy the blue color of the Fehling solution. If it contains more than this, it must be diluted with water to 5 or 10 times its volume, measuring accurately the water added and mixing thoroughly.

The Fehling solution after dilution is heated to boiling, and the sugar solution run in from a burette until the blue color has been destroyed, leaving the liquid colorless above the red precipitate. If too much sugar has been added it begins to turn yellowish. The amount of sugar

is ascertained most quickly by making two determinations: first, a rough one, then one which is made more carefully. Make the first by running in the sugar solution, 2 or 3 cubic centimeters at a time, as long as the blue color is well marked, then 1 cubic centimeter at a time, heating to boiling after each addition. It can be learned by this first test within 1 or 2 cubic centimeters how much will be required. Then rinse out the beaker, take again 10 cubic centimeters of the Fehling solution, diluted as before; heat to boiling and run in at once within 1 cubic centimeter of the necessary amount of the sugar solution. Bring it to a boil. Then add the sugar solution a few drops at a time, heating after each addition, until the blue color has just been decolorized.

Since 10 cubic centimeters of the Fehling solution is decolorized by 0.05 gramme of glucose, the amount of the sugar solution or urine which has been used from the burette must have contained 0.05 gramme of glucose. Read the volume which has been poured from the burette, and calculate the percentage of sugar in the original solution. If this has been diluted with water the amount in the dilute solution must be multiplied by the number of times it was diluted. Remember that however much of the sugar solution may have been used to destroy the blue color, it contained 0.05 gramme of sugar. For example, if the amount used was 10 cubic centimeters, there would be 0.005 gramme of glucose in 1 cubic centimeter; that is, in 1 gramme of solution. In 100 grammes there would be 0.5 gramme of glucose, or 0.5 per cent.

Lactose (Milk-sugar: $C_{12}H_{22}O_{11} + H_2O$).

Lactose is found in the milk of all mammals and occasionally during pregnancy in the urine. It can be obtained

from the milk by crystallization after the removal of the nitrogenous constituents.

It is a crystalline substance, soluble in water, with a faint sweetish taste. With pure yeast it does not ferment. By the action of certain other ferments, however, it undergoes alcoholic fermentation, with the production at the same time of lactic acid, forming the drinks known as "koumiss," when made from mares' milk, and "kephyr" when from cows' milk. The ordinary souring of milk is due to the formation of lactic acid from the lactose by micro-organisms.

Milk-sugar gives with many reagents the same results as glucose. It can be distinguished from glucose by its not fermenting with yeast and by its having a less strong power of reduction. It is unable to reduce cupric compounds to cuprous in acetic acid solutions (Barfoed's test).

EXPERIMENT 33.—Preparation of milk-sugar. Dilute 200 cubic centimeters of milk with 800 cubic centimeters of water, and add very cautiously not more than 0.1 per cent. of acetic acid to precipitate the casein (when enough has been added the liquid is nearly clear). Filter. Boil the filtrate and filter off the coagulated albumin. Evaporate the filtrate upon a water-bath to a syrup and allow it to stand until the sugar has crystallized out. It may be purified by recrystallizing it.

EXPERIMENT 34.—Test the milk-sugar with Trommer's, Fehling's, and the phenyl-hydrazin tests, and notice that the results are similar to those obtained with glucose.

EXPERIMENT 35.—Try Barfoed's and the fermentation tests as made with the glucose, and observe that the results are negative.

EXPERIMENT 36.—Boil the milk-sugar solution with a little hydrochloric acid, neutralize, and try the fermenta-

tion or Barfoed's test. The lactose has been changed to glucose and this will now ferment, forming carbon dioxid and alcohol.

Sucrose (Cane-sugar: $C_{12}H_{22}O_{11}$).

Cane-sugar is found in plants, not in the animal kingdom. It has no reducing power and does not respond to the tests where such a reducing action occurs, such as Trommer's, Fehling's, and Boettger's. It is decomposed by heating with acid into a molecule of glucose and one of fructose.

Experiment 37.—Apply Trommer's test to a solution of cane-sugar. It gives no results.

Experiment 38.—Boil a solution of cane-sugar with a little sulphuric or hydrochloric acid, neutralize the solution, and prove that it contains glucose.

Experiment 39.—The "invert-sugar" which results from the decomposition of cane-sugar by acids can be separated into glucose and fructose by adding to 10 parts 6 parts of calcium hydrate and 50 parts of water. Both sugars form calcium compounds. That with glucose, being liquid, can be pressed out of the fruit-sugar compound, which can then be decomposed, the fruit-sugar being set free, by the addition of oxalic acid as long as a precipitate is produced. Filter and obtain the fructose by the evaporation of the filtrate.

Maltose (Malt-sugar: $C_{12}H_{22}O_{11}$, H_2O).

Experiment 40. Preparation of Pure Maltose.—One hundred grammes of starch are to be mixed with 500 cubic centimeters of cold water as thoroughly as possible, then heated on a water-bath until it makes a paste. Make a watery extract of malt at 40° C. from 6 or 7 grammes of dry malt. When the starch-paste has cooled down to 60° or 70°, add the malt-extract and keep it at this temperature for an hour. When the starch has

been converted to maltose the liquid becomes thin and watery. Then boil, filter, and evaporate to a syrup upon the water-bath. Dissolve the maltose from the residue with small portions of 90-per-cent. alcohol. Distill the alcohol off from this solution, and evaporate to a syrup. Let this stand until it crystallizes. This may be hastened by the addition of a little crystallized maltose, which can be prepared by evaporating a few drops of the solution in a thin layer on a piece of glass. It may be purified by recrystallizing from methyl alcohol.

EXPERIMENT 41.—Try the test with phenyl-hydrazin on the maltose. Try also Trommer's test. It responds to both.

THE FATS.

The fats occur in both plants and animals. When pure they are colorless, odorless, and tasteless. They are insoluble in water and have a lower specific gravity. They dissolve in hot alcohol more easily than in cold, and are easily soluble in ether, gasolin, or benzol. The fats mix with water when the two are shaken violently together, but they soon separate, the fats going to the top. If, however, something like soap or a solution of albumin is added to the mixture which will form a coating around the minute globules of fat, they are prevented from reuniting, and form an emulsion,—that is, a mixture of small fat-globules with the liquid, not a solution. It is destroyed by anything which will remove the coating, the fat separating again from the liquid.

The fats are composed of three elements: carbon, hydrogen, and oxygen. They contain a much smaller percentage of oxygen than the carbohydrates, the hydrogen and oxygen not being in the proportion to form water. When the fats are kept at the temperature of superheated steam or subjected to the action of the pancreatic ferment, they take up water and are split into two compounds:

glycerin, on the one hand, and one or more of the fatty acids, on the other. Thus, stearin gives

$$\underset{\text{stearin}}{C_3H_5(C_{18}H_{35}O_2)_3} + \underset{\text{water}}{3H_2O} = \underset{\text{glycerin}}{C_3H_5(OH)_3} + \underset{\text{stearic acid}}{3C_{17}H_{35}CO_2H}.$$

They may be considered then as made up of glycerin and a fatty acid less water.

This splitting up of the fat-molecule is called saponification. It occurs when fats become rancid. It can be also effected by boiling the fat with a caustic alkali. Here, instead of the free fatty acid being left, it unites with the alkali to form a salt. These metallic salts of a fatty acid are the soaps:—

$$\underset{\text{stearin}}{C_3H_5(C_{18}H_{35}O_2)} + \underset{\text{alkali}}{3KOH} = \underset{\text{glycerin}}{C_3H_5(OH)_3} + \underset{\text{soap}}{3C_{17}H_{35}CO_2K}.$$

The soaps of the alkalies are soluble in water, the potassium compound being hygroscopic and forming soft soap. The sodium compound forms a hard soap. The compounds of the heavy metals with the fatty acids are insoluble, and can be formed by adding a solution of their salts to a soap solution. The lead soap or lead plaster used in medicine is made by heating lead oxid with one of the fats. The soluble soaps can be thrown down from their solutions by saturation with a neutral salt. If a strong acid is added to a soap solution the soap is decomposed, the metal uniting with the strong acid and the fatty acid being set free as an insoluble substance:—

$$\underset{\text{soap}}{C_{17}H_{35}CO_2K} + \underset{\text{acid}}{HCl} = KCl + \underset{\text{fatty acid (stearic)}}{C_{17}H_{35}CO_2H}.$$

These fatty acids which enter into the most of the animal and vegetable fats are one of the unsaturated series: oleic acid ($C_{17}H_{33}CO_2H$); and two of the saturated series: stearic acid ($C_{17}H_{35}CO_2H$) and palmitic acid ($C_{15}H_{31}CO_2H$). Besides these acids—which constitute, by far, the larger part of those present in fats—there are found in some cases certain of the lower members of the saturated series, such as butyric ($C_3H_7CO_2H$), capronic ($C_5H_{11}CO_2H$), caprylic ($C_7H_{15}CO_2H$), and capric ($C_9H_{19}CO_2H$), which occur in butter.

The acids which enter into the fats resemble the latter in many of their properties. They differ from them in having a slight acid reaction, the fats being neutral. They can also be distinguished by their not giving the irritating odor of acrolein as the fats do when they are heated. This is produced by the decomposition of glycerin, either in a fat or when heated alone. It is most easily obtained by adding before heating some substance which will assist in removing the water. The chemical change is

$$C_3H_5(OH)_3 - 2H_2O = C_3H_4O.$$

The free acids can be neutralized by even weak alkalies, forming the soaps.

The animal and many of the vegetable oils are true fats, differing only in that they are liquids at ordinary temperatures instead of solids. We must, however, distinguish between these and the essential or volatile oils which are found in plants, but which are not fats. The fats produce spots on paper which are not volatile and do not disappear on standing. The essential oils will disappear when left exposed to the air. The mineral oils belong to an entirely-different class of compounds, and do not contain oxygen.

The fats are named from the acid which they contain. Thus the compound of stearic acid is called stearin; of palmitic acid, palmitin; and of oleic acid, olein. Sometimes the prefix *tri* is used with these names, as tristearin, etc. They differ principally in their melting-points, olein being a liquid at ordinary temperatures; palmitin and stearin, solids, the former melting more easily than the latter. In the animal body these fats are usually mixed, the consistence of the fat varying with the composition. Thus, the fat of the ox, or tallow, is a firmer solid than the fat of the hog, or lard, because it contains less of the olein. The animal oils contain more olein than stearin and palmitin, consequently they melt at temperatures below the ordinary ones, and are liquids. Human fat contains 67 per cent. to 80 per cent. of olein.

Experiment 42.—Try the reaction of a fresh fat, like lard or olive-oil, with a piece of litmus-paper. It is neutral; but, if the fat has been standing some time and has become rancid, it may be slightly acid.

Experiment 43.—Try the solubility of a few drops of olive-oil in a test-tube of water. It mixes when shaken violently, but soon separates at the top on standing. Add now a few drops of a soap solution and shake again. The liquid becomes milky and the fat does not separate. If the oil is not fresh it may be necessary to add a few drops of sodium carbonate to neutralize the free acid.

Experiment 44.—Examine a drop of the emulsion so formed under the microscope. It will be found to consist of a great number of minute globules the size being smaller the more thoroughly the liquid is shaken. They are kept apart by the thin film of soap which covers each one.

Experiment 45.—Add a small amount of hydro-

chloric acid and shake. The soap will be decomposed and the fat will collect at the top, as at first.

EXPERIMENT 46.—Try the solubility of a fat in ether, chloroform, or gasolin, avoiding carefully the vicinity of a flame. It is easily soluble.

EXPERIMENT 47.—Try the solubility of tallow or lard, first in cold, then in warm, alcohol. It is readily soluble in the warm alcohol and separates on cooling in the crystalline form. The crystals can be examined with the microscope.

EXPERIMENT 48.—Show that the fats are non-volatile by placing a little upon paper and warming it over a flame. It does not disappear.

EXPERIMENT 49.—To about 10 grammes (11 cubic centimeters) of olive-oil add 20 cubic centimeters of 10-per-cent. potassium hydrate solution. Boil the mixture, gently stirring, meanwhile, until the odor of the oil has largely disappeared and it appears homogeneous and no oil separates when a few drops are poured into water. This may require fifteen minutes to half an hour. Add water as it evaporates, to keep the original volume. The product is a mixture of potassium soap and glycerin.

EXPERIMENT 50.—Convert a portion of the soap into the sodium or hard soap by adding some saturated salt solution and allowing it to stand until cold. It will dissolve on warming.

EXPERIMENT 51.—To another portion add a calcium solution. A calcium soap is formed which is insoluble in water. It is this compound which is produced by the action of soap on "hard water." Many of the heavy metals give similar compounds. Try it with solutions of iron, lead, copper, etc.

EXPERIMENT 52.—To the remainder of the potassium

soap solution add sulphuric acid slowly until it is plainly acid to test-paper. The fatty acids are set free as insoluble substances, the glycerin remaining in solution. Filter out the acids by means of a wet filter-paper, through which they will not pass. Save the filtrate for the extraction of the glycerin. Wash out the sulphuric acid with distilled water until the wash-water is no longer acid, and try the reaction of the fatty acids with litmus-paper. They are acid to litmus.

EXPERIMENT 53.—Allow the fatty acids to stand until the water has drained off or dry them by the aid of filter-paper. Heat them in a dry test-tube until they commence to decompose, and see if they give the irritating odor of acrolein. If the acids were washed clean this should not appear. Try the same test on any of the fats, and the odor will be given. It is best produced by adding before heating a little acid potassium sulphate.

EXPERIMENT 54.—Neutralize the first filtrate from the acids, which contains the glycerin, by adding a little powdered chalk, then evaporate the mixture to complete dryness on a water-bath. Extract the glycerin from the powdered mass by alcohol and evaporate this on the water-bath. If the glycerin is to be further purified this residue should be extracted with absolute alcohol and the alcoholic filtrate evaporated. The thick liquid is the glycerin, as is shown by the taste.

EXPERIMENT 55.—Make the acrolein test upon the water-free glycerin by heating in a dry tube with some crystals of potassium bisulphate. The glycerin is decomposed, giving the acrolein odor, and showing that the odor when obtained from fats is due to the glycerin radical, and not to the acid part.

EXPERIMENT 56.—Show that olive-oil is not a simple fat, but a mixture, by cooling it to the freezing-point either by natural cold or by a freezing mixture. It separates into

two parts: one crystalline, consisting mostly of palmitin, and the other olein, which remains liquid.

The Lecithins.

The lecithins are found in nearly all animal and vegetable cells. They are very abundant in the brain and nerves and in the yelk of eggs. They are sometimes called phosphorized fats. The formula of one of those most common in the animal body is $C_{44}H_{90}NPO_9$, and the composition is probably

$$C_3H_5 \left\{ \begin{array}{l} C_{18}H_{35}O_2 \\ C_{18}H_{35}O_2 \\ O \\ O= \end{array} \right\} P \left\{ \begin{array}{l} OH \\ O—C_2H_4—N \end{array} \right\} \begin{array}{l} (CH_3)_3 \\ OH \end{array}$$

It is therefore a fat where for one stearic acid radicle has been substituted the group

$$——PO_3\overline{OH}\ C_2H_4\ N(\overline{CH_3})_3\ OH.$$

In others, instead of the stearic acid radicle we may have the oleic or palmitic radicle. Like other fats, they can be decomposed or saponified. They then give phosphoric acid, a fatty acid, and a base, cholin:—

$$HO\ C_2H_4\ N(CH_3)_3\ OH.$$

Lecithin is a soft, waxy substance, which swells in water to a pasty mass. This, under the microscope, has the form of oily drops or threads, the so-called "myelin" forms. It resembles nuclein in the readiness with which it unites with albuminous substances. It is found in the yelk of the egg in an unstable union with vitellin.

EXPERIMENT 57. PREPARATION OF LECITHIN.—In the following work the student should remember that ether and petroleum-ether are very inflammable.

Separate the albumin of an egg as completely as possible from the yelk. Place the yelk in a cylindrical, glass-stoppered bottle, add two or three times its volume of ether, and shake the bottle until they are well mixed. Allow it to stand until the ether above becomes clear, and then decant the latter into a distilling flask. Repeat this extraction several times, when most of the coloring matter should have been dissolved. Mix the portions of ether and distill off the ether. The residue contains the lecithin mixed with fats, cholesterin, and coloring matters. Dissolve this in petroleum-ether and filter. Pour the filtrate into a separatory funnel, add about one-fourth its volume of 75-per-cent. alcohol, and shake. When the two liquids have separated draw off the alcohol, which contains most of the lecithin. Repeat this extraction with alcohol several times and unite the alcoholic solutions. Distill off the remainder of the petroleum-ether from these, and let the solution stand several days in a cool place. A precipitate of cholesterin and other impurities will form, from which the solution is to be decanted through a filter. Boil the filtrate with a little animal charcoal to decolorize it, and filter. Evaporate at a temperature of 50° to 60° to a syrupy consistency. Cool this and dissolve in ether. If it does not dissolve completely, filter it. Evaporate the ether, when the lecithin remains nearly pure. If desired it can be purified further by dissolving in as small an amount as possible of warm absolute alcohol and placing this in a freezing mixture of —5° to —15°, when the lecithin crystallizes out. It should be filtered in the cold.

EXPERIMENT 58.—Place a little lecithin in water and examine with the microscope. Notice the myelin forms.

EXPERIMENT 59.—Warm this mixture with water and notice that after a time the lecithin turns brown and the reaction becomes acid from decomposition.

EXPERIMENT 60.—Mix a little lecithin with dry, powdered potassium nitrate in a small porcelain crucible, and warm, at first gently, then, after deflagration, until the dark color has disappeared. After cooling dissolve in water and test for phosphoric

acid by nitric acid and ammonium molybdate. At once or after warming a yellow precipitate will appear.

THE PROTEINS.

The protein compounds constitute the greater part of the solid matter of the blood, muscles, nerves, and other organs of the animal body. The urine, tears, and perspiration, in a normal condition, never contain more than a trace. The proteins contain carbon, hydrogen, nitrogen, oxygen, and usually sulphur. A few contain phosphorus and a few others iron. When heated they are charred, giving off water, inflammable gases, and ammonia, at the same time emitting a strong odor similar to that of burnt horn or wool. Upon further ignition they leave an ash, though whether this was originally a part of the protein molecule has not been decided. They are often spoken of simply as the nitrogenous constituents of the body or the food, although not all of the nitrogenous compounds found there belong to this class.

The proteins are very complex substances with a high molecular weight, and it is probably owing to this fact that they are so easily decomposed, as is seen by the putrefaction which sets in soon after life has ceased. To the large molecule, too, is probably due the inability of most of them to pass through a parchment or animal membrane.

General Properties of the Proteins.

Experiment 61.—Burn a small piece of dry albumin or other protein compound on a piece of porcelain or platinum foil, or on a wire. Notice that it turns black from

the presence of carbon. Observe the characteristic odor. On continued heating it will all disappear except the mineral matters, or ash.

EXPERIMENT 62.—Mix a few fragments of dry albumin with an excess of powdered soda-lime and heat the mixture in a dry test-tube. Test the vapors which escape for ammonia, both by the odor and by their action, on a piece of red litmus-paper. The ammonia proves that the albumin contained nitrogen.

EXPERIMENT 63.—Make a solution of lead hydrate by adding sodium hydrate to a small amount of lead acetate solution until the precipitate first formed has dissolved. Add to it a protein compound, like albumin, and boil. The presence of sulphur in the protein compound is shown by the dark-colored lead sulphid, which it forms by uniting with the lead.

EXPERIMENT 64.—Very small quantities of sulphur may be shown by changing it to a sulphid and testing for the latter with sodium nitroprussid. By this means it can be found in a single hair. Shut off the air from a Bunsen burner and, after turning it low, cover the hair with sodium carbonate and hold it on a wire in the middle of the flame. Allow the substance to fuse, being careful to keep it in the yellow flame to prevent oxidation. Then dissolve the mass in a few drops of water in a procelain dish. Add to the solution a very small crystal of sodium nitroprussid. The presence of sulphid is shown by the production of a purple or violet color, which is destroyed by an excess of the nitroprussid.

EXPERIMENT 65.—Test a solution of egg-albumin or any other albuminous substance to see if it will pass through a dialyzer. Only the peptones will pass through the membrane. The biuret test can be used to detect them (Experiment 66).

CLASSIFICATION OF THE PRINCIPAL PROTEIN COMPOUNDS.

- Proteins
  - Albuminous substances
    - Albumin.
    - Globulin.
    - Albuminates
      - Acid albumin.
      - Alkali albumin.
    - Albumose.
    - Peptones.
    - Fibrin.
    - Coagulated albumin.
  - Proteids
    - Mucin.
    - Hæmoglobin.
    - Nucleoalbumin.
    - Nuclein.
  - Albuminoids
    - Keratin.
    - Elastin.
    - Collagen.
    - Gelatin.
    - Etc.

ALBUMINOUS SUBSTANCES.

These are sometimes called proteins, though it is more convenient to reserve this name for the whole class, including also the proteids and albuminoids. They form the principal part of the protoplasm which is found in animal and plant cells. The composition of the molecule and even the exact formula of the different members of the group is uncertain. They are known to be very complex, those which have been most studied having several hundred atoms in a molecule. They differ somewhat from each other in composition, but their constituents usually lie within the following limits:—

Substance.	Approximation.	Average of Most Analyses.
C	50.0 to 55.0 per cent.	52 per cent.
H	6.5 to 7.3 per cent.	7 per cent.
O	20.0 to 23.5 per cent.	23 per cent.
S	0.3 to 2.2 per cent.	2 per cent.
N	15.0 to 18.0 per cent.	16 per cent.

Phosphorus is sometimes found in less amounts than 1 per cent.

A few of the albuminous substances have been obtained in an imperfectly crystallized form, but most of them are amorphous. They differ in their solubilities and are classified largely upon this basis. The peptones will diffuse through an animal membrane, but they do not pass through rapidly.

The albuminous substances, like some other organic compounds which do not belong to this class, are thrown out of solution when to the solution certain neutral salts are added until it is saturated. Ammonium sulphate will precipitate all but the peptones and perhaps a few of the albumoses. Magnesium sulphate and sodium chlorid will precipitate many of them.

When the albuminous substances are heated with water, many of them are coagulated, passing into an insoluble modification. The temperature at which this takes place is called the coagulation-point. This is a different one for most of the different substances, and may be used in their identification and separation. It may vary, however, from the presence of other substances. It may be raised, prevented, or the coagulation made incomplete by alkalies or by some organic acids, like acetic acid. Coagulation is favored and the coagulation-point is lowered in the presence of neutral salts or small amounts of a mineral

acid. The concentration of the solution also may make it vary. Through coagulation the nature of albuminous substances is altered and they acquire other properties. By the action of alcohol albuminous compounds are precipitated, at first in an unaltered form; but if the alcohol is strong and acts for some time they are coagulated, and are then insoluble in water.

Coagulation, when spoken of with respect to the protein compounds, must be distinguished from precipitation, which it resembles. When albumin is coagulated—*e.g.*, by boiling, by mineral acids, or by the continued action of strong alcohol—it becomes insoluble in water. It may be precipitated without being coagulated by ammonium sulphate or by not too large an amount of alcohol and still retain its original properties, being soluble again upon the addition of water.

Some of the albuminous compounds are coagulated by the action of ferments; for example, the fibrin, which is so formed from the blood or lymph.

Albuminous substances are easily decomposed by the action of the putrefactive bacteria, the nitrogen and sulphur uniting with hydrogen to form hydrogen sulphid, and ammonia, or, these two together, ammonium sulphid. Other nitrogen compounds are also formed, like the amido acids which contain the amido group, NH_2, such as leucin and tyrosin. Indol is also one of the nitrogenous putrefactive products.

Many of the albuminous substances are precipitated by the mineral acids, but upon standing with an excess of the acid, or more quickly by heating, they are dissolved, going into acid albumins. Many will also form insoluble compounds with salts of the heavy metals, such as mercury, copper, and lead. With copper in an alkaline solution they

give a blue or purple color and upon boiling with an excess of nitric acid a yellow, which becomes more reddish upon being rendered alkaline. Millon's reagent, which gives a red with all compounds containing a benzol nucleus united with an hydroxyl group, produces the same color with albuminous compounds.

GENERAL REACTIONS OF THE ALBUMINOUS SUBSTANCES.

The tests may be made upon any albuminous compound; for example, egg-albumin.

EXPERIMENT 66.—Make the solution alkaline with sodium hydrate and add a few drops of a dilute cupric sulphate solution. A blue or purple color results. An excess of the copper solution must be carefully avoided, as it may produce a blue color when no protein compound is present. (Biuret test.)

EXPERIMENT 67.—Add a small quantity of concentrated nitric acid to the albumin solution and heat to boiling. A yellow color is produced which becomes orange red when the liquid is made alkaline with sodium hydrate or ammonia. (Xanthoproteic reaction.)

EXPERIMENT 68.—Make the solution of albumin acid with acetic acid, then add at least an equal volume of a saturated solution of ammonium sulphate, and heat to boiling. Most albuminous compounds are thrown down as a white precipitate.

EXPERIMENT 69.—Acidify the solution with acetic acid and add a few drops of potassium ferrocyanid. A white precipitate is formed.

The two last reagents fail in case of the peptones.

CLASSIFICATION OF THE ALBUMINOUS SUBSTANCES ACCORDING TO THEIR MOST IMPORTANT REACTIONS.

	Solubility.	*Coagulation on Heating.*	*Precipitation by*
Albumins.	In water.	Coagulate.	Saturation with neutral salts.
Globulins.	In diluted solutions of neutral salts.	Coagulate.	Neutral salts.
Albuminates { *Acid.*	Acids or alkalies; not in cold water.	Non-coagulable.	Neutralizing.
Albuminates { *Alkaline.*		Non-coagulable.	Neutralizing.
Albumoses.	Most in water.	Non-coagulable.	Neutral salts, as $(NH_4)_2SO_4$, etc.
Peptones.	Water.	Non-coagulable.	Not by $(NH_4)_2SO_4$ or neutral salts.
Fibrin.	Insoluble in water.	Coagulates.	
Coagulated Albumin.	Insoluble in water.		

EXPERIMENT 70.—Add an excess of Millon's reagent and boil. A red color is produced with all albuminous compounds except antipeptones.

EXPERIMENT 71.—Many of the albuminous compounds are also precipitated by (1) trichloracetic acid; (2) metaphosphoric acid; (3) alcohol or ether; (4) from solutions acidified with HCl or HNO_3 by potassium mercuric iodid or phosphotungstic acid; (5) from acetic acid solutions by picric or tannic acid; (6) from weakly alkaline solutions by salts of mercury and some of the other heavy metals. Try these.

No reaction is characteristic in itself; therefore in testing for these bodies a number of tests should be tried.

ALBUMINS.

The albumins are soluble in water. They are not precipitated by dilute acids or alkalies. They are precipitated by saturation with ammonium sulphate and by some of the salts of the heavy metals, such as mercuric chlorid and salts of copper, silver, and lead. For this reason albumin is successfully used as an antidote for poisoning by many of these metallic salts. By the action of acids it is converted into acid albumin and by the caustic alkalies into alkali albumin.

There are several different varieties of albumin usually named from their sources, such as the serum-albumin of the blood; egg-albumin; and the albumin of milk, or lactalbumin. We know that these are not the same substances from their physiological action, as well as from some chemical and physical properties. If, for example, egg-albumin be injected into the circulation it passes in a short time into the urine. Serum-albumin injected in the same manner does not pass unaltered through the kidneys.

Serum-albumin is more easily dissolved by an excess of mineral acid than egg-albumin. They differ also in their coagulation-temperatures.

Albumin may be obtained for the experiments by dissolving the commercial dry egg-albumin or serum-albumin in cold water, filtering if it is not clear. From fresh eggs it can be prepared by separating the albumin from the yelk, being careful not to mix them. Then beat the albumin, to break up the membranes in it, mix with twice its volume of water, and filter through a piece of unbleached muslin.

Purified egg-albumin may be prepared by the following method:—

EXPERIMENT 72.—Separate the white from the yelk and cut it for some time with scissors or beat it up to a strong foam. Allow this to subside; filter or press it through clean, unsized muslin; mix it with an equal volume of water, and, after the precipitate has subsided, filter it. Saturate the filtrate at about 20° with very finely powdered magnesium sulphate, added in small portions with continual stirring. When it is saturated filter off the precipitated globulins and dialyze the filtrate until a portion gives only a faint turbidity with barium chlorid. The solution will increase in volume during the operation. It should be concentrated in flat dishes at 40° to 50°, again dialyzed, and finally evaporated to dryness at the above temperature. It still contains 1 to 3 per cent. of ash.

EXPERIMENT 73.—To obtain purified serum-albumin the blood must be drawn into a dry vessel and the fibrin coagulated by beating. Filter this out through muslin and separate the corpuscles from the serum by a centrifugal machine or by allowing it to stand in a tall cylinder until the corpuscles have settled. Horse-blood, if obtainable, is best for this purpose. Then draw off the clear serum with a siphon, dilute it with three volumes of a saturated solution of ammonium sulphate, and add gradually, stirring continually, finely powdered ammonium sulphate until the liquid is saturated. Filter off the precipitate, which contains serum-albumin and paraglobulin, and wash it with a saturated

solution of ammonium sulphate. It will be purer if it is dissolved in a small quantity of water and the precipitating and washing are repeated. Dissolve the precipitate while it is still moist in the smallest possible quantity of water and dialyze it. The paraglobulin separates out as a white flocculent precipitate, while the albumin remains in solution. Filter out the globulin and wash it with water. It is insoluble in water, but soluble in dilute solutions of neutral salts. It is precipitated by neutral salts like ammonium sulphate, magnesium sulphate, or sodium chlorid. Its sodium chlorid solution coagulates at about 75°.

If the solution of serum-albumin contains no globulin, it will not be made turbid by adding to a small portion magnesium sulphate to saturation. In that case it is to be carefully neutralized with ammonia, the salts dialyzed out, the contents of the dialyzer filtered, if necessary, and evaporated at 40° to a small volume. The solution should be precipitated by alcohol and immediately filtered. Press out the excess of alcohol as much as possible, and wash out the remainder with ether.[1] Allow the ether to escape by stirring the albumin in a mortar.

Experiment 74.—Try the effect of various reagents upon the coagulation-temperature of albumin by placing about 10 cubic centimeters of an albumin solution in a number of test-tubes, first filtering if necessary to obtain a clear liquid, and adding to (1) sodium chlorid; (2) concentrated sodium chlorid and a few drops of acetic acid; (3) a few drops of dilute hydrochloric acid; (4) sodium hydrate; (5) a few drops of a solution of sodium carbonate; (6) nothing. Set the test-tubes in a beaker of water and heat to boiling, observing which coagulate first. It will be found that acids and neutral salts favor coagulation and that alkalies hinder it or prevent it altogether.

Experiment 75.—Test solutions of serum-albumin and egg-albumin with an excess of nitric acid without heat

[1] This must be done away from the vicinity of any light or fire.

and also by warming, and notice that the serum-albumin is more easily soluble. Try the same with concentrated hydrochloric acid.

EXPERIMENT 76.—Show that a solution of egg-albumin forms an insoluble compound when added to solutions of mercury, silver, or copper.

GLOBULINS.

The globulins are distinguished from all the other albuminous substances by being soluble in dilute salt solutions, but insoluble in water. From this solution they are precipitated by diluting freely with water or by removing the salt by dialysis. They can, in this manner, be separated from the albumins which remain in solution. The globulins are, as a rule, mostly precipitated by saturating the solution with neutral salts, like sodium chlorid. They are coagulated by heating with water. By the action of dilute acids they are converted into acid albumin. A common example is myosin, which is found in muscle.

EXPERIMENT 77.—Prepare myosin from lean meat by chopping about an ounce finely, then stirring it well with cold water to remove the albumin. Filter through muslin, and repeat the treatment with water until it is white or nearly so. Squeeze out most of the water and treat the residue with a 10-per-cent. solution of ammonium chlorid. For thorough extraction it should stand several hours, but enough for testing can be obtained by stirring for five minutes. Filter through muslin, then through paper. The filtrate contains the globulin (myosin).

EXPERIMENT 78.—To a beaker full of pure water add a little of the solution. The myosin is precipitated.

EXPERIMENT 79.—Heat some of the globulin solution. It is coagulated.

EXPERIMENT 80.—To the solution of myosin add enough hydrochloric acid to make it contain 0.1 per cent. After it has stood a few hours syntonin, an acid albumin, has been formed. If the change is complete it does not coagulate upon heating. If there is a coagulum it is some of the unchanged myosin. In this case filter it out and test the filtrate for the acid albumin by carefully neutralizing it with dilute sodium hydrate. The acid albumin which is formed from the globulin by the dilute acid is precipitated.

EXPERIMENT 81.—Try the general tests for albuminous substances (xanthoproteic, biuret, Millon's). The myosin responds to all.

THE ALBUMINATES.

These are called also acid and alkali albumins. They are formed from the albuminous substances by the action of acids or alkalies. They are soluble in water which contains a small amount of acid or alkali, but are not in neutral solution. Consequently they are precipitated when their solution is neutralized. They are not coagulated by boiling. The alkali albumin has the properties of an acid, giving a slight acid reaction. Similarly the acid albumin has a slight alkaline reaction. They are named from the manner in which they are produced, and not according to their reaction.

ACID ALBUMIN.

EXPERIMENT 82.—Prepare by adding dilute HCl to a solution of egg-albumin till it contains 0.1 per cent. of the acid. Allow it to stand an hour, at about body-temperature, then filter and neutralize with very dilute sodium hydrate, being careful not to add an excess, as this would dissolve the precipitated acid albumin. Wash the precipitate in water.

EXPERIMENT 83.—Notice that the acid albumin is

soluble in acids, though insoluble in water. Make a solution in dilute HCl and boil. It is not coagulated.

EXPERIMENT 84.—Make acid albumin by the action of strong HCl, HNO_3, or acetic acid on serum-albumin or egg-albumin, warming if necessary. It is formed very quickly. Show that it gives the xanthoproteic reaction and biuret reaction, though it is not precipitated by boiling.

ALKALI ALBUMIN.

EXPERIMENT 85.—Prepare from a solution of albumin by warming with an alkali, like sodium hydrate.

EXPERIMENT 86.—The solid alkali albumin can be obtained by adding strong sodium hydrate, drop by drop, to the white of an egg, stirring continually. No more must be added after it has become gelatinous, as it will then dissolve. Wash the solid in cold water, in which it is insoluble, though it is soluble without difficulty in warm water.

EXPERIMENT 87.—Observe that the alkali albumin is soluble in dilute acids or alkalies. An excess of acid may precipitate it.

EXPERIMENT 88.—Dissolve some of the solid substance in hot water or use the solution obtained from Experiment 85. Add a few drops of phenolphthalein, which gives a red color, showing that the solution is alkaline. Add slowly dilute acid until the red color has just disappeared, when the solution will be neutral. The alkali albumin is precipitated. If, now, more acid is added, the precipitate dissolves. It is, consequently, like the acid albumin, precipitated by neutralizing.

EXPERIMENT 89.—Show that alkali albumin, like acid albumin, is not coagulated by boiling.

EXPERIMENT 90.—The thoroughly washed, solid alkali albumin when pressed upon moist litmus-paper reddens it, and its solution in calcium hydrate has an acid reaction, if no excess of the latter is present. To obtain this result, however, great care is necessary to free it from the alkali used in its preparation.

ALBUMOSES AND PEPTONES.

The peptones are the final products of the digestive action of pepsin upon the albuminous compounds. The albumoses are intermediate products between the albuminous compounds and the peptones. They may both be formed by the putrefaction of albuminous substances.

The albumoses give the general reactions of the albuminous substances, but they do not, like the former, coagulate on boiling. They are distinguished from the peptones by giving a precipitate with nitric acid or potassium ferrocyanid acidified with acetic acid. They are also precipitated by saturating their solution with ammonium sulphate or sodium chlorid, then acidifying. They diffuse with difficulty through an animal membrane.

The peptones do not give any of these reactions, but respond to the general ones of albuminous compounds, especially the biuret test, where the resulting color is a reddish pink. They do not coagulate on boiling, and, unlike the other albuminous substances, will pass through a parchment or animal membrane. They cannot be precipitated by ammonium sulphate or with potassium ferrocyanid. Tannin or alcohol precipitates them from their solutions.

A number of different classes have been described, two of the principal ones being those designated by the prefix *anti*, which resist the action of ferments and are not easily decomposed further. The *hemi* compounds are more easily decomposed. Thus we have antialbumoses and hemialbu-

moses, and antipeptones and hemipeptones. The other classes differ principally in their solubilities.

In the dry state the albumoses form an amorphous powder. The peptones also have an amorphous form, but are extremely hygroscopic, dissolving, to a resinous mass, in the water which they absorb from the air. Their taste is unpleasant.

Experiment 91. Preparation of Albumoses.—Make a solution of hydrochloric acid containing 0.2 per cent. Add to it a few grains of pepsin and a small handful of blood-fibrin. Let it stand at a temperature of about 38° C. until the fibrin has dissolved. Neutralize carefully with dilute sodium hydrate, heat to boiling, filter, concentrate the filtrate on the water-bath to a small volume, and precipitate the albumoses by adding sodium chlorid until the liquid is saturated. The precipitate, which is a mixture of different albumoses, is filtered off, and to the filtrate is added acetic acid. This precipitates an albumose, called deuteroalbumose. The filtrate from this will give another albumose as a precipitate upon saturation with ammonium sulphate.

Experiment 92.—Dissolve some of the albumose in water and show that it is precipitated by potassium ferrocyanid in a solution acidified with acetic acid.

Experiment 93.—Show that a solution of albumose in water is not coagulated by neutralizing, boiling, nor by the heating after addition of nitric acid to the solution, though the latter may precipitate them in the cold (distinction from albumin and albuminates).

Experiment 94. Preparation of Peptone.—Digest blood-fibrin in a neutral solution with a watery extract from a chopped pancreatic gland at about body-temperature. If it is allowed to stand many hours, add a few crystals of thymol to prevent putrefaction. Boil after solution has taken place, filter, concentrate by boiling, and saturate while boiling with ammonium sulphate to precipitate the albumoses. Filter these out, first by muslin, then by filter-paper. The solution may be used for testing or the ammonium sulphate may be removed largely by evaporation and crystallization. The remainder can be removed by adding first

barium hydrate, then barium carbonate, and boiling, until a portion of the filtrate gives no precipitate with barium chlorid.

EXPERIMENT 95.—Commercial peptone may be used for testing. Test the solution of peptones by adding first sodium hydrate, then not more than two or three drops of copper sulphate (biuret test). A red or pink color is produced, which is characteristic of the peptones. If too much of the copper solution is added, the color is bluish.

EXPERIMENT 96.—Place some of the solution in a dialyzer and leave it an hour, then test the solution outside for the presence of peptones. They will be found to have passed through the membrane, although they do not dialyze rapidly.

EXPERIMENT 97.—Show that tannic acid precipitates peptones in a neutral solution.

EXPERIMENT 98.—Show that the peptones are not precipitated by potassium ferrocyanid acidified with acetic acid, as are the albumoses if they contain none of the latter.

FIBRIN.

Fibrin is formed as a gelatinous mass when fresh blood coagulates. If the blood is beaten during its coagulation the fibrin collects together into strings as elastic as caoutchouc, and remains so as long as it is moist. It can be freed from the blood coloring matter by washing with water or a salt solution. It is coagulated by heating with water.

COAGULATED ALBUMIN.

The albumins may be converted into the coagulated form by heating with water or by the continued action of strong alcohol. This is insoluble in water, but can be dissolved by caustic alkalies or by heating with the strong

mineral acids, being thereby converted into alkali or acid albumins.

The Proteids.

This class of substances is more complex than the albuminous substances. They can all be decomposed into albuminous substances, on the one hand, and, on the other, bodies which are not albuminous. Thus the albuminous compound is, in hæmoglobin, united with the hæmatin molecule; in the nucleins, with phosphoric acid, etc. They can be considered, then, as unions of an albuminous substance with some other substance. Most of them are coagulated by boiling.

The Mucins.

Mucins are found in some of the secretions of the body, especially in those of the mucous membrane and saliva, and also as a constituent of the tendons and umbilical cord. In their composition they resemble the albuminous substances, but contain less nitrogen. Their characteristic property is that when boiled with a dilute mineral acid they are decomposed, giving two substances: an albuminous compound and a compound containing little or no nitrogen and having the power of reduction, as is shown by its changing cupric hydrate to cuprous hydrate in an alkaline solution. By this they can be distinguished from all similar albuminous compounds. There are several kinds of mucin, although as yet they are not well differentiated from one another.

The mucins are colloid substances, insoluble in pure water, but soluble in small amounts of dilute alkalies, such as calcium hydrate. They are mucilaginous and can be drawn out into threads. They are precipitated by the addition of acetic acid, if neutral salts are absent. They

are not coagulated by boiling, but give many of the reactions of the albumins. Like the nucleoalbumins, they are acid in reaction.

There have been sometimes included with the mucins a class of substances which resemble them in being decomposed by acids into albuminous substances and substances with the power of reduction, but which are not precipitated by acetic acid. They are more properly called mucoids. Such are the pseudomucin, found in ovarial liquids; chondromucoid of cartilage, and a few others.

Experiment 99. Preparation of Mucin.—Mince finely a submaxillary gland of an ox and extract it with water. Filter and add to the filtrate strong hydrochloric acid until the liquid contains 0.15 per cent. of acid, avoiding an excess. The mucin is at first precipitated, but dissolves again upon stirring. Then add two or three volumes of water, which will precipitate it. Separate it from the liquid by filtration or decantation and repeat the dissolving and precipitation as before. Wash with water and, if the dry substance is desired, with alcohol and ether.

Experiment 100.—Try the solubility in calcium hydrate, and precipitate from this solution by acetic acid.

Experiment 101.—Boil mucin for some time with dilute hydrochloric acid, and, after making the liquid alkaline, show by Fehling's test that there is a reducing body present.

Experiment 102.—Show that solutions of mucin in an alkali will give the biuret test.

The Nucleoalbumins.

The nucleoalbumins occur widely distributed in the animal and vegetable kingdoms, forming one of the principal constituents of protoplasm. They are found especially

in the cell, but sometimes in the secretions, such as the milk, which contains casein. Some authors, however, include in the class of nucleoalbumins only those found in the cell, and exclude such as the casein of milk and the vitellin of eggs.

Chemically they are composed of the same elements as albumin, but contain, in addition, phosphorus and sometimes iron. They resemble in their properties the globulins and alkali albumins. They differ, however, in containing phosphorus. They are also insoluble in neutral salt solutions, unlike the globulins, and differ from the alkali albumins by being decomposed by the action of gastric juice into an albumin, which is digested, and an indigestible phosphorus compound called nuclein. The nucleoalbumins may be considered, then, as compounds of a nuclein and an albuminous substance.

The nucleoalbumins are insoluble in water or dilute acids. They can be dissolved in alkaline solutions. They have the properties of dibasic acids, as is shown by the fact that the solution in an alkali where not too much of the alkali has been used have a slight acid reaction. The acid reaction is also shown by their setting free the carbonic acid of carbonates. The nucleoalbumins can be precipitated from their alkaline solutions by acidifying. The acid removes the alkali with which they are united and sets the nucleoalbumin free as an insoluble substance. This may be shown in the precipitation of the casein in milk.

The nucleoalbumins are soluble in strong acetic acid or an excess of hydrochloric acid, being at the same time decomposed, nuclein being set free and the albumin being converted into acid albumin. They are also decomposed and coagulated by suspending the free nucleoalbumin in water and boiling.

In general, being compounds of the albuminous substances, the nucleoalbumins respond to the same tests.

EXPERIMENT 103. PREPARATION OF CASEIN.—Dilute about 100 cubic centimeters of milk with 400 cubic centimeters of water, and precipitate the casein until the liquid above is nearly clear by adding acetic acid drop by drop, avoiding an excess. Filter and wash with water. If fat-free substance is desired, it must be extracted in an extraction-apparatus with ether. For many tests this is not necessary.

EXPERIMENT 104.—Test the casein for nitrogen and sulphur in the same manner as albumin was tested (Experiments 62 and 63).

EXPERIMENT 105.—Test it also for phosphorus by mixing about a gramme of the dry substance with equal parts of sodium carbonate and potassium nitrate and fusing in a porcelain crucible. After cooling, dissolve the mass in water, acidify strongly with nitric acid, and add ammonium molybdate. A yellow precipitate, at once or after warming, shows the presence of phosphoric acid.

EXPERIMENT 106.—Make the same tests upon casein as were made upon alkali albumin and notice that similar results are obtained.

EXPERIMENT 107.—If the rennin ferment is at hand or can be prepared, test with it a solution of casein in lime-water. The casein is changed into paracasein calcium, which is only slightly soluble in water (cheese).

EXPERIMENT 108.—Pulverize some freshly precipitated casein in a mortar with water and calcium carbonate. The casein acts like an acid in that it sets free the carbonic acid from the carbonate.

EXPERIMENT 109.—Make a gastric juice by adding a

few grains of pepsin to 0.2-per-cent. HCl[1] and digest in it for some time at the temperature of the body some casein. The nucleoalbumin is decomposed into two substances: an albumin, which is dissolved, and a nuclein, a compound rich in phosphorus, which remains. Filter out the nuclein, and test the filtrate with the biuret test for the albuminous compound.

The Nucleins.

The nucleins occur partly combined with albuminous substances as nucleoalbumins, partly free in the nucleus of the cell. They are composed of phosphoric acid united with an albumin, or sometimes in addition with a nuclein base, such as adenin, guanin, xanthin, or hypoxanthin. Besides the elements found in the albumin they contain phosphorus and sometimes iron. The iron in the hæmoglobin is probably obtained by the animal organism from such compounds as these. We have no absolute proof that the common salts of iron can be assimilated by the animal organism. One of the nucleins which may furnish iron to the animal body is hæmatogen, found in the yelk of eggs.

The nucleins may be divided into two classes:—

1. Those which, when acted on by hot acids or alkalies, take up water and give, as decomposition-products, phosphoric acid and an albumin. They have been called *paranucleins*. Such a one is contained in casein. It can be set free by digesting by gastric juice the albumin with which it is united. Hæmatogen is also of this class, and probably furnishes the iron for the hæmoglobin of the young bird.

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[1] This strength of acid can be obtained by diluting concentrated hydrochloric acid with 150 times its volume of water.

2. The second class is decomposed by dilute acids or alkalies, giving, besides phosphoric acid and an albumin, one or more of the nuclein bases, also called xanthin bases, xanthin, guanin, etc. This class is found principally in the nucleus of the cell and has been called nuclear, or nucleus, nucleins.

The nucleins are not attacked by gastric juice, and this is used to separate them from the albuminous compounds, which can be digested by it. They are insoluble in water and dilute acids, but soluble in alkalies. They give the biuret and Millon's tests in consequence of the albumin which they contain.

EXPERIMENT 110.—Mix 50 grammes of compressed or brewers' yeast with 200 grammes of water, and allow the yeast to subside. Pour off the water and to the residue add 0.5-per-cent. potassium hydrate solution; stir, and, after waiting a few minutes to dissolve the nucleins, filter and acidify with hydrochloric acid. Filter out the precipitated nucleins, wash with HCl, then hot alcohol. Dry over sulphuric acid. Try solubility in acids and alkalies, also biuret and Millon's tests. The residue gained by fusion with sodium carbonate and potassium nitrate gives a yellow precipitate with ammonium molybdate, showing the presence of phosphoric acid.

A few of the nucleins, both animal and vegetable, contain iron, and these are the principal source of the iron in the animal body, our food-materials containing no inorganic compounds of iron. Hæmatogen is an example of such nucleins. It is found in the yelk of hen's eggs united with an albuminous substance, which can be split off by digestion.

The iron in such organic combinations as nuclein does not respond to the ordinary chemical tests until the compound has been decomposed by chemical agents or other means. Reagents like ammonium sulphid and potassium ferrocyanid decompose it very slowly, whereas they act immediately upon the inorganic compounds of iron and not at all upon hæmatin. The nucleins which contain iron are important from their being probably the origin of the animal iron compounds.

Hæmatogen.

Experiment 111.—Prepare from the yelk of an egg. Shake the yelk in a wide-mouth, glass-stoppered bottle with two or three times its volume of alcohol; allow it to stand and when it has settled pour off the alcohol. Repeat this operation twice, then extract in the same manner or, better, in an extraction apparatus with ether until the residue is white. Digest this in artificial gastric juice (made as in Experiment 151). The nuclein, hæmatogen, remains.

Experiment 112.—Dissolve a portion in ammonia. Test for iron by ammonium sulphid. At first there is no color, but after a time the solution turns greenish and, in twenty-four hours, black, as the iron is gradually set free from the compound. In the same manner test with potassium ferrocyanid. The Prussian blue is formed slowly, differing thus from its production with the ordinary iron salts.

Experiment 113.—Add to the nuclein some hydrochloric acid, then, after neutralizing with ammonia, test for iron as before. The acid has decomposed the nuclein, so that the tests are obtained immediately.

The Albuminoids.

The albuminoids are found in the insoluble form, mostly in the bones of the body or the parts which are used for protection. They resemble the albuminous substances, giving many of the same products when they are decomposed, differing, however, in other respects. They are not easily attacked by the reagents which dissolve and decompose albuminous compounds. Only the collagen and its derivative, gelatin, with possibly elastin, are digestible.

Collagen.

This substance is found in the animal body in the connective and cartilaginous tissues, tendons, and bones. That from the bones was formerly called ossein. It is com-

posed of the same chemical elements as the albumins, but contains a little more oxygen. It is probably an oxidation-product of some of the albumins. It is insoluble in water, but by boiling with water it is converted into gelatin or glue. By the action of tannic acid collagen is changed to a form which does not putrefy. This is the action which takes place when leather is tanned.

Gelatin.

Gelatin may be considered as the hydrate of collagen, as it is formed by the union of collagen with water. It can be changed back into collagen by heating for some time at 130°. It swells up in cold water and dissolves when the water is warmed. On allowing the solution to cool it gelatinizes, or becomes a semisolid. It acts in this respect oppositely to albumin, which is soluble in the cold, but becomes a solid by the action of heat.

After it has been boiled a long time with water it is decomposed, and does not gelatinize on cooling, peptone being formed. The sulphur of gelatin is united in the molecule in a different manner from that of the albumin molecule, as is proved by its decomposition-products.

Gelatin is decomposed by the gastric juice, giving products similar to those from albumin. It has been found, however, that it cannot take the place of the albuminous materials of food, though it is of value when used with them.

Gelatin does not give all the reactions of the albuminous substances, although it does give the same results with some of them. Like the albuminous compounds, it gives a purple color with the biuret test; it is precipitated by picric acid, by mercuric chlorid in the presence of sodium

chlorid and hydrochloric acid, by tannic acid in the presence of sodium chlorid, and by saturation with ammonium sulphate. On the other hand, it is not coagulated by boiling; it is not precipitated by mineral acids; and it does not give a brown color when warmed with an alkaline solution of lead, as albumin does, the sulphur being apparently too firmly united to be split off and form lead sulphid. It does not give the xanthoproteic reaction when pure.

EXPERIMENT 113*a*.—Prepare collagen from bone by dissolving out the mineral constituents with dilute hydrochloric acid (HCl, 1 part: water, 8 parts) until they are flexible, then wash out the acid. Notice that the collagen is not soluble in dilute acid nor cold water. To remove all the albumins it may be necessary to soak awhile in 5-per-cent. NaOH solution, then wash again.

EXPERIMENT 114.—Convert the collagen into gelatin by warming it with water. Notice that it gelatinizes upon cooling the solution.

EXPERIMENT 115.—Boil a portion of the solution for some time, and notice that it is thus decomposed, so that it will not form a jelly upon cooling.

EXPERIMENT 116.—Test a portion of the gelatin solution with the biuret test. It gives a purple color like albumin.

EXPERIMENT 117.—Show that it is precipitated by tannic acid in the presence of NaCl.

EXPERIMENT 118.—Show that gelatin is not precipitated by nitric or other mineral acids, but is by saturation with ammonium sulphate and also by mercuric chlorid in the presence of HCl and NaCl.

EXPERIMENT 119.—Show that gelatin contains sulphur by heating with dry sodium carbonate in the reducing flame, then testing with sodium nitroprussid as in the case of albumin (Ex-

periment 64), but that it gives no black sulphid of lead when heated in a solution of lead acetate in an excess of sodium hydrate (Experiment 63).

Elastin.

Elastin occurs in the connective tissues,—in the cervical ligament (ligamentum nuchæ) very abundantly. It differs from most of the proteins in containing no sulphur, except possibly some that is loosely combined with the molecule. In the moist state it is very elastic: when dry it is hard and brittle. By the action of the digestive ferments it is decomposed into bodies called elastoses, similar to the albumoses. It gives the general reactions of the proteins.

Experiment 120.—Prepare elastin from the cervical ligament of an ox by cutting it into thin slices and boiling it for several days to remove the gelatin. Boil then with 1-per-cent. potassium hydrate for several hours, afterward with water. Repeat the boiling with 10-per-cent. acetic acid: then let it stand twenty-four hours in 5 per-cent. hydrochloric acid. Wash with water, boil with 95-per-cent. alcohol, and extract with ether to remove the fat. For the complete removal of the latter more than a week may be required.

Experiment 121.—Try the tests used for sulphur in albuminous substances, and see that it is not present.

Keratin.

The keratins are the chief constituent of the horny part of the epidermis, of hair, horns, nails, feathers, etc. They contain a large amount of sulphur,—4 or 5 per cent.,—a part of which is so loosely united that it is set free by boiling water. It is owing to this sulphur that the salts of lead, silver, and some other metals act as hair-dyes, the sulphur uniting with the metal to form a dark-colored sulphid. The keratin is not at all attacked by the gastric or pancreatic juices. It is decomposed when heated, giving the odor of burnt horn. It is insoluble in water, and gives the xanthoproteic and Millon's reactions.

Experiment 122.—Prepare keratin by boiling some horn-shavings with water and then digesting them in succession in a

dilute solution of pepsin containing 0.2-per-cent. HCl, and a trypsin solution. Wash with water, alcohol, and ether.

EXPERIMENT 123.—Boil keratin with sodium hydrate, filter, and test the filtrate for sulphur by lead acetate. It produces a black precipitate of lead sulphid.

EXPERIMENT 124.—Show that keratin responds to the xanthoproteic and Millon's tests.

FERMENTATION.

By fermentation we mean the decomposition of an organic substance into simpler and more stable molecules, the agent which causes the change being itself unaffected. The agents are living organisms or are formed by such organisms. The living ferments—such as the yeast-plant or bacteria—are called the organized ferments. They have the power of reproduction and are composed of cells. The non-living ferments are known as the unorganized ferments, or enzymes. They may be excreted by the organized ferments or secreted by living cells, which latter is the case with the digestive ferments. They are not reproductive and act outside of the cell where they were formed. The difference between the two classes may be seen in the action of bakers' yeast on a dilute cane-sugar solution. The yeast-cell excretes an enzyme called invertin, which acts upon the sugar, producing glucose. The latter is fermented by the yeast-cell, giving carbon dioxid and alcohol. The yeast (living ferment) may be killed by adding chloroform or some other protoplasmic poison to the liquid, but the enzyme is not affected. In this case glucose is produced, but no further fermentation is noticed.

The enzymes of the animal cell exist in the cells in an inactive condition, called zymogens, but become active after standing exposed to the atmosphere or being brought in contact with certain chemical compounds. The enzymes

contain nitrogen and from their properties are apparently identical with the proteins. They are indiffusible and soluble in glycerin and in water. They can be mechanically removed, without decomposition, from their solutions by forming precipitates therein, to which they adhere, also by saturating the solutions with ammonium sulphate. A low temperature stops their action and they are all killed below 100° if moisture is present. Most enzymes act best at about 38° C. The ferment is not destroyed, but its action is stopped, by a large accumulation of its own products.

The organized ferments contain albumin, fat, cellulose, and some inorganic salts. They survive a high temperature better than the enzymes, but are killed at 100° except certain spore forms. Moisture is necessary for them to act.

As is the case with the enzymes, a sufficient amount of their products stops their further action. This is the effect of alcohol upon the yeast-plant.

Experiment 125.—Add a little yeast to a dilute solution of cane-sugar in water and keep it for some time at the body-temperature.

Test the solution with Trommer's test. The copper compound is reduced by the glucose and lævulose which have been formed from the sucrose by the invertin of the yeast. If allowed to stand a long time the glucose is changed by the yeast to alcohol and carbon dioxid.

Experiment 126.—Stir a little compressed yeast into lukewarm water in a test-tube, and after it has stood a few minutes, add a few drops of chloroform and mix thoroughly by shaking. Fill the rest of the tube now with dilute cane-sugar solution and let it stand inverted for twenty-four hours in a warm place, as in Experiment 31.

The organized ferment (yeast) is killed by the chloroform, so that there is no alcoholic fermentation with a formation of carbon dioxid.

Experiment 127.—Test the liquid with Trommer's test. It responds to the test, showing that the enzyme (invertin) is not destroyed, but has decomposed the cane-sugar, as before, to glucose and lævulose.

Experiment 128.—Separate the mucous membrane from the muscular coating of a pig's stomach; chop finely and allow to stand several hours with two or three times its weight of dilute phosphoric acid (1 per cent.). Filter and to the filtrate, which contains the pepsin, add lime-water until the reaction is alkaline. The calcium phosphate which falls carries down the pepsin with it. Filter and dissolve the precipitate in dilute HCl. Place in a dialyzer, changing the water outside frequently. The acids and salts diffuse out, leaving the pepsin inside the dialyzer, as can be proved by adding it to 0.2-per-cent. HCl and seeing that it will digest fibrin.

Experiment 129.—Test a part of the precipitated pepsin which has been obtained with the calcium phosphate for nitrogen. This may be done, after washing with water, by drying the precipitate, still mixed with calcium phosphate, then mixing with twice as much soda-lime and testing in a dry tube. The nitrogen is converted into ammonia, which may be recognized by the odor and by its action on red litmus-paper.

Experiment 130.—To a part of the dialyzed-pepsin solution obtained in Experiment 128 add finely powdered ammonium sulphate, stirring meanwhile as long as it dissolves. The pepsin is precipitated like the albuminous compounds. Filter, dissolve the precipitate in a 0.2-per-cent. HCl solution, and show that it will digest fibrin.

Experiment 131.—Collect some saliva in a test-tube, place the latter in a beaker of water, and raise the temperature of the water to 65° or 70° C. Keep it at this point for five minutes. Then let a little of it stand a few minutes with a starch solution, testing afterward with Trommer's

test. No glucose is produced, the ferment, ptyalin, having been destroyed by the heat.

EXPERIMENT 132.—Collect a considerable quantity of saliva, and put it into two tubes. Quickly cool one nearly to freezing and warm the other to body-temperature. Add to these an equal amount of starch solution. Allow the action to proceed for five minutes, then raise them both to the boiling-point to stop fermentation. Determine the amount of sugar formed by Fehling's quantitative test (Experiment 32), or, approximately, by the subnitrate of bismuth test (Experiment 29). There has been little or no fermentation in the cold liquid.

EXPERIMENT 132*a*.—In the same manner cool another portion of the saliva and, after ten minutes, warm to body-temperature and show by its decomposing starch that the ferment is not destroyed by the cold.

EXPERIMENT 133. PREPARATION OF LACTIC ACID BY FERMENTATION.—In 150 cubic centimeters of boiling water dissolve 30 grammes of cane-sugar and add about 30 milligrammes of tartaric acid. Let the solution stand two days, then add 40 cubic centimeters of sour milk and about half a gramme of old cheese. After the addition of 15 grammes of zinc oxid allow the mixture to stand ten days at a temperature of 40° to 50°, with repeated stirrings. At the end of that time heat to boiling, filter while hot, and allow to cool. Zinc lactate will crystallize out on cooling if the solution is sufficiently concentrated. If it is not it should be allowed to evaporate to a smaller volume. It may be purified by recrystallizing. The acid can be obtained by dissolving the lactate in water and decomposing by hydrogen sulphid gas. Filter off the zinc sulphid, evaporate the filtrate to a syrup, and after it is cold extract the acid by dissolving it in ether.[1] When the ether has evaporated the acid will remain. It may be preserved for testing in the gastric juice tests.

[1] Remember that the vapors of ether are extremely inflammable.

Experiment 134. Preparation of Butyric Acid.—Make a mixture as for the preparation of lactic acid, or use a part of that mixture. Allow the fermentation to go on as before, but for three or four weeks. In that time bubbles of hydrogen and carbon dioxid appear; and, after removing the zinc, butyric acid is found in the ether. It can be identified by its acid reaction and characteristic odor.

Experiment 135. Preparation of Kephyr.—The preparation of the ferment requires several days. First stir the kephyr-grains in a little lukewarm water and decant the latter. Then cover them with ten times their weight of milk which has been boiled and cooled. Let them stand for twenty-four hours at about 20° C., then pour off the milk, rinse the grains with a little water, and repeat the digestion with milk for twenty-four hours as before. This should be continued until the milk has an acid reaction and the grains rise to the upper part of the liquid, which may require five or six days. After this the grains may be allowed to stand with a moderate quantity of milk, which becomes filled with the organized ferment. At the end of twenty-four hours this is filtered through muslin, and the filtrate added to ten or fifteen times its volume of previously boiled and cooled milk. The whole is poured into a clean strong bottle, the cork is tied in, and it is allowed to stand two or three days at a temperature below 15° C., when the fermentation is complete. The grains on the muslin can be used an indefinite number of times in the same manner.

Experiment 136.—Test the acid reaction of the kephyr by litmus-paper (lactic acid); also show the presence of alcohol by the iodoform test (Experiment 31).

THE SALIVA.

The saliva is a mixture of the secretions of the parotid, submaxillary, and sublingual glands with that of the glands of the membrane of the mouth. Its reaction is normally faintly alkaline. The mixed saliva is a colorless, more or less viscid liquid, often opalescent. On standing it deposits calcium carbonate as a film on the surface. Examined microscopically it is seen to contain epithelial cells

from the membrane of the mouth, air-bubbles held by viscid liquid, and salivary corpuscles which resemble the lymph-corpuscles. Bacteria are abundant.

The normal mixed saliva contains

I. Inorganic	Carbonates, Chlorids, Sulphates, Nitrites	of	Magnesium, Calcium, Potassium, Sodium.
	Sulphocyanid of potassium.		
II. Organic	Albumin.		
	Mucin.		
	Ptyalin.		

Nitrites and sulphocyanids are often absent. The ptyalin has the power to convert boiled starch into dextrin, maltose, and glucose. It is not able to penetrate the granule of the unboiled starch, or does so very slowly, differing in this respect from the corresponding ferment of the pancreas. Its presence can be detected by mixing the saliva with about ten times its volume of a solution of boiled starch, keeping it awhile at body-temperature, and after a few minutes testing for sugar. The ptyalin acts best at about 40°, and is, therefore, not the same ferment as the diastase of malt, which decomposes starch most rapidly at a temperature of 55°. Ptyalin is destroyed by acids, even as dilute as the 0.2-per-cent. hydrochloric acid of the gastric juice. It is, however, probable that it acts for some time in the stomach before the acid penetrates the mass of food in a large enough quantity to stop the fermentation.

The secretion is influenced by the nervous system. It can be increased by mechanical means, like chewing a

pebble or a piece of rounded glass in the mouth; by chemical action, such as touching the tongue with a crystal of tartaric acid or filling it with the vapor of ether or acetic acid; or by electrical excitation. In collecting saliva for testing it should be acomplished without trying to hasten its flow by suction with the tongue, as this increases the amount of secretion from the mucous glands and so dilutes the secretion of the salivary glands.

The composition of the saliva is changed by certain pathological conditions. The amount is diminished in all febrile conditions, also in diabetes and often in nephritis. It is increased by the action of some medicinal substances, like the mercury compounds, pilocarpine, and others; also by anything which causes irritation or inflammation of the glands. Urea has been found in it abundantly during nephritis. The reaction becomes acid in fevers and in diabetes, and this sometimes happens also after long-continued talking.

Experiment 137. — Collect for examination some saliva by letting it flow into the mouth without swallowing. Excite the flow if necessary by taking the vapor of ether or acetic acid into the mouth. Notice the indication of mucin in the viscidity, as well as the lasting foam after beating it with a glass rod.

Experiment 138.—Let a portion stand exposed to the air, and notice the separation of calcium carbonate as a white film or turbidity.

Experiment 139.—Test a portion for potassium sulphocyanid by adding to it a very dilute solution of ferric chlorid. A red color indicates the sulphocyanid. This is immediately decolorized by the addition of a few drops of mercuric chlorid solution.

Experiment 140.—Test for nitrites with a few drops

of a starch solution acidified with a little dilute sulphuric acid and containing a small amount of potassium iodid. A nitrite immediately gives a blue color.

EXPERIMENT 141.—Test for albuminous substances by the xanthoproteic test.

EXPERIMENT 142.—Test for mucin by adding to the clear saliva acetic acid drop by drop. The mucin separates in white, stringy flakes. They may be washed with water and tested by the mucin reactions.

EXPERIMENT 143. PREPARATION OF PTYALIN.—Collect a large amount of saliva and acidify with phosphoric acid. Then add milk of lime until the liquid has a faint alkaline reaction. The phosphoric acid is precipitated as calcium phosphate and carries the ptyalin down with it. Filter and allow the water to drain off without washing. Place the precipitate in a beaker and add not more water than the original amount of the saliva. Stir it well and filter. This removes the ptyalin from the calcium phosphate, and it goes into the filtrate. Add to the filtrate an excess of alcohol. A white precipitate will separate, which is ptyalin mixed with inorganic salts. To free it from these, dissolve in a little water and precipitate with absolute alcohol. Repeat this operation if necessary. Dry it over sulphuric acid.

EXPERIMENT 144.—Test the aqueous solution of ptyalin. It is not precipitated by nitric acid like albumin, nor does it give the xanthoproteic reaction. It can be precipitated after a time by basic lead acetate, the filtrate being without action on starch.

EXPERIMENT 145.—To some boiled starch-paste cooled to about body-temperature add a small quantity of the ptyalin solution or of saliva, and after standing at that temperature for a short time test it with Trommer's test for glucose. The latter should be present.

EXPERIMENT 146.—Try the same experiment with unboiled starch. Glucose is not formed, or, if at all, only after a long time and in small amounts.

EXPERIMENT 147.—Try the effect of dilute acids by

diluting 1 cubic centimeter of concentrated hydrochloric acid with 150 cubic centimeters of water, then adding to the saliva an equal volume of the dilute acid. This makes the acidity of the whole about the same as that of the gastric juice. Let the acidified saliva act on boiled starch as before. No glucose is produced.

THE GASTRIC JUICE.

The gastric juice, secreted by the glands of the stomach, differs from the other digestive fluids in having an acid reaction. It is a clear, thin liquid containing, as inorganic constituents, principally the chlorids and phosphates of the alkalies and of calcium and magnesium. There is more hydrochloric acid than can unite with the bases, and this must consequently be in the free state. The most important of the organic substances are two enzymes, or unorganized ferments: pepsin and rennin.

The acidity of the gastric juice is caused principally by the free hydrochloric acid, but may be at times due to the acid phosphates, or to the organic acids: lactic, butyric, and acetic. The hydrochloric acid and acid phosphates are present in the normal juice. The lactic acid may be found in the first stages of digestion, especially when the food contains much of the carbohydrates, but is not normally found after digestion has proceeded more than half an hour. The acetic and butyric acids are not normally present.

The free hydrochloric acid appears to be formed from the chlorids which are taken with the food. Its formation has been explained as due to the action of disodium phosphate upon calcium chlorid and also to the decomposition of sodium chlorid by a weak acid, like carbonic acid.

There are, however, many points which have not yet been made sufficiently clear. A free acid is necessary for the digestion of the nitrogenous foods by the pepsin, and this is one of the offices of the hydrochloric acid. Recent researches have indicated that one of its most important functions is the prevention of fermentation in the stomach. The mineral acids have antiseptic powers even in such dilution as that of the hydrochloric acid in the gastric juice; that is, from 0.2 to 0.3 per cent. Such a solution will, for several days, prevent putrefaction in animal matter, like chopped meat, which would otherwise soon commence to decay. It will also destroy the bacteria of many infectious diseases, though some of these are not affected when in the form of spores.

The effects of increased fermentation are seen in certain pathological conditions where the secretion of hydrochloric acid is diminished or stopped. They are especially noticeable in the case of food containing large quantities of carbohydrates. The sugar which is formed by the saliva may be changed by the ferments into different acids:—

$$\underset{\text{glucose}}{C_6H_{12}O_6} = 2\underset{\text{lactic acid}}{C_3H_6O_3}.$$

By further fermentation the lactic acid undergoes this change:—

$$2C_3H_6O_3 = \underset{\text{butyric acid}}{C_3H_7CO_2H} + 2CO_2 + 2H_2.$$

The glucose may be fermented to alcohol:—

$$\underset{\text{alcohol}}{C_6H_{12}O_6 = 2C_2H_5OH} + 2CO_2$$

and this be converted to acetic acid:—

$$C_2H_5OH + O_2 = \underset{\text{acetic acid}}{CH_3CO_2H} + H_2O.$$

The so-called "heart-burn," which is one of the accompanying symptoms of gastric fermentation is caused by the eructations of gas carrying the acids up into the throat, where they cause irritation of the mucous membrane. The methods of treatment based upon administration of hydrochloric acid, creasote, or other antiseptic substances is not for the purpose of removing the acids, but to stop the fermentation. Treatment with alkaline substances like magnesia or sodium bicarbonate neutralizes the acids, but does not prevent fermentation.

The acid phosphates may be present in the normal gastric juice. When present they increase the acidity of the juice or cause an acid reaction in the absence of free acids. An example is the potassium compound, KH_2PO_4, which is found in the stomach after meat has been eaten.

The pepsin can be obtained from the gastric juice or mucous membrane of the stomach in a number of ways. Like most of the animal ferments, it can be extracted from the membrane by glycerin, and this glycerin solution can be preserved for any length of time. If it is to be used immediately, water containing about 0.2-per-cent. hydrochloric acid can be employed for extraction. If the dry substance is desired, it may be thrown down with finely-divided precipitates of other substances, as is the case with many of the ferments.

The pepsin thus obtained is probably not the pure substance. It is a white or yellowish-white, amorphous powder or scale when dried. It is hygroscopic in the air and has a slightly saline or acidulous taste, with no offensive odor. It is soluble in about 100 parts of water, but more easily on the addition of hydrochloric acid. Pepsin belongs to the proteins. Its molecular composition is unknown.

Pepsin is inactive in neutral or alkaline liquids, but in slightly acid fluids it dissolves coagulated albuminous

compounds, with the formation of albumoses and peptones. It acts most rapidly with hydrochloric acid, though others may be used instead. The best strength of acid for the purpose varies with the kind of material to be digested from 0.1 per cent. to 0.3 per cent. of hydrochloric acid. Pepsin from warm-blooded animals digests best at 38°. It is destroyed by heating the solution. Its action is hindered by the presence of the products of digestion, but if these are removed as fast as they are formed it will change to the soluble form several thousand times its weight of albuminous material.

The second ferment of the gastric juice, rennin, is always present in human gastric juice under normal conditions. It exists in the mucous membrane in the form of a zymogen, which is sometimes inactive until it is set free by an acid. Hence if it is extracted by water it may not give its characteristic reaction. This is especially true in the case of birds or fish. This characteristic reaction is the coagulation of milk or casein in a neutral or faintly-alkaline solution in which calcium salts are present. It does not give the albumin reactions when in a pure state. Rennin is more easily destroyed by heating its solution than is pepsin. The methods of obtaining it are similar to those employed with pepsin.

The gastric juice acts only upon the nitrogenous constituents of the food. The albuminous substances are first changed into acid albumin by the free acid; then this is decomposed by the pepsin and hydrochloric acid, forming albumose, which passes into peptone. Since the process is a continuous one, all these products may be found in the stomach at the same time, though in the first stages the acid albumin is in excess and at the last the peptones. The connective tissues are digested by the gastric juice,

though the proteolytic ferment of the pancreas does not dissolve them. The membranes which surround the fat-cells are also dissolved, setting the fat free. Thus the food is changed into chyme: a pulpy mass which can be readily penetrated by the intestinal fluids. In the first stages of digestion the saliva may continue to act on the starchy materials, and during the first fifteen to twenty minutes there is a formation of lactic acid, which disappears after this time. Milk is coagulated, partly by the acid, partly by the rennin. The absorption of the peptones commences in the stomach, but the digestion is not completed here, the chyme passing through the pylorus into the intestine.

Experiment 148. Preparation of Pepsin.—Separate the mucous membrane of a pig's stomach from the muscular tissue. After rinsing it with water chop it finely. Make a dilute solution of hydrochloric acid by the addition of 1 cubic centimeter of concentrated acid to 150 cubic centimeters of water. This will contain about 0.2 per cent. of the pure acid. Extract the pepsin from the chopped membrane by means of this dilute acid. To obtain a strong solution of pepsin it is best to let it stand for twenty-four hours in a cool place, though it will be found in the solution in a very short time. The rennin, which is dissolved at the same time, is destroyed by the pepsin on standing. Filter the liquid through muslin, then, if necessary, through paper. This solution can be used directly for digestive experiments or for preparing the purified pepsin.

Experiment 149.—Purified pepsin can be obtained by precipitating it with some substance which is thrown down as a finely-divided precipitate. To effect this, neutralize the hydrochloric acid solution of pepsin in the preceding experiment and acidify with phosphoric acid, or extract the pepsin from the membrane with water acidified slightly with phosphoric acid. Precipitate by

adding lime-water or milk of lime until the liquid is alkaline. Filter off the precipitate, which contains the pepsin mixed with calcium phosphate. Dissolve in water with the addition of hydrochloric acid. Remove the salts by dialysis. The pepsin does not pass through the membrane. A purer form is obtained by repeating the solution in acid and precipitation with lime-water or by precipitating with alcohol before dialyzing. If the dry substance is desired, the drying must be at a low temperature. This may be done over sulphuric acid in a vacuum.

EXPERIMENT 150.—To make a pepsin solution which may be preserved, though it is somewhat impure, chop the mucous membrane finely, as in the previous methods, and after squeezing it in a piece of cloth to remove the water as far as possible, cover it with two or three times its volume of glycerin and let it stand for a week. Filter it through a piece of muslin, pressing out the glycerin. This gives a permanent solution which can be used at any time for digestive experiments.

EXPERIMENT 151.—Make an artificial gastric juice by preparing a 0.2-per-cent. solution of hydrochloric acid as in Experiment 148, and adding to this a small quantity of the glycerin solution of pepsin. The solution made directly with the dilute acid can be used if it is fresh. To this add a small handful of washed fibrin. If this is not at hand, boiled egg-albumin may be substituted for it, though in this case the digestion is slower. Warm the mixture carefully to about body-temperature and keep it at this point until the albuminous substance has dissolved. Notice that the edges first become transparent, then are dissolved. Too high heat will destroy the ferment. The fibrin should be nearly digested in half an hour. It may be left for several days without danger of putrefaction provided it contains 0.2 per cent. of the free acid.

When the fibrin has nearly or quite disappeared boil

the liquid to coagulate any unchanged albuminous compound, and filter. The filtrate contains the products of digestion: acid albumin, albumoses, and peptones. The proportion of each varies with the time of digestion. The amount of peptones is usually small until the pepsin has acted for some time.

EXPERIMENT 152.—Precipitate the acid albumin by neutralizing carefully with very dilute sodium hydrate. Filter it out and test as in Experiment 83.

EXPERIMENT 153.—From the filtrate precipitate the albumoses by saturating the solution with ammonium sulphate by the aid of heat. Filter and test the precipitated albumoses as in Experiments 92 and 93.

EXPERIMENT 154.—The filtrate from the albumoses contains the peptones. Test it according to the tests used in Experiments 95 and 98.

EXPERIMENT 155. PREPARATION OF RENNIN.—Extract the chopped mucous membrane with 0.2-per-cent. hydrochloric acid, as in Experiment 148. Both rennin and pepsin go into solution. Stir the membrane well in the acidified water, but do not let it stand a very long time, as the rennin is digested in an acid solution by the pepsin. Neutralize carefully, add a small amount of magnesium carbonate, and shake well. The pepsin adheres to the carbonate and can be filtered out with it. Shake the filtrate with another portion of magnesium carbonate, filter, and repeat the operation until the pepsin is removed, which can be shown by the failure of an acidified portion of the filtrate to dissolve fibrin. The filtrate containing the rennin should coagulate milk in a few minutes.

EXPERIMENT 156.—In order to purify the rennin precipitate it with basic lead acetate and filter. Wash the precipitate, then suspend it in water, and acidify slightly with sulphuric acid. Filter and to the filtrate, which contains the rennin, add a solution of stearin soap. The latter is decomposed by the acid, stearic acid being set free as an insoluble precipitate. This carries

the rennin with it. Filter and place the precipitate, with a small amount of water, in a glass-stoppered funnel. Add ether, shake, and after the ether has separated above the watery solution draw off the latter. The fatty acids from the decomposed soap have remained in the ether, leaving the rennin dissolved in the water.

Experiment 157.—Test the solution or a specimen of normal or artificial gastric juice for rennin by exactly neutralizing, then adding it to an equal volume of milk in a test-tube. Place the tube in a beaker of water at body-temperature. If rennin is present the casein will be coagulated in twenty or thirty minutes.

A solution of casein may be used instead of milk, but in order to make it coagulate a small amount of a calcium salt must be added.

Experiment 158. Valuation of Pepsin (U. S. P. Process).—Prepare three solutions:—

(*A*) To 294 cubic centimeters of water add 6 cubic centimeters of dilute (10 per cent.) HCl. (*B*) In 100 cubic centimeters of *A* dissolve 0.067 gramme of the pepsin. (*C*) To 95 cubic centimeters of *A* at a temperature of 40° C. add 5 cubic centimeters of *B*. The resulting 100 cubic centimeters of liquid will contain 2 cubic centimeters of dilute hydrochloric acid, 0.00335 gramme of the pepsin to be tested, and 98 cubic centimeters of water.

Immerse and keep a hen's egg during fifteen minutes in boiling water; then remove it and place it in cold water. When it is cold separate the coagulated albumin and rub it through a clean sieve having thirty meshes to the linear inch. Reject the first portion passing through the sieve. Weigh off ten grammes of the second, cleaner portion, place it in a flask of a capacity of about 200 cubic centimeters, then add one-half of solution *C*, and shake well so as to distribute the coherent albumin evenly through the liquid. Then add the second half of solution *C*, and shake again, guarding against loss. Place the flask in a water-bath kept at a temperature of 38° to 40° C. for six hours, shaking it gently every fifteen minutes. At the expiration of this time the albumin should have disappeared, leaving at the most only a few thin, insoluble flakes. Trustworthy results, particularly in comparative trials,

will be obtained only if the temperature be strictly maintained between the prescribed limits, and if the contents of the flasks be agitated uniformly and in equal intervals of time.

The relative proteolytic power of pepsin stronger or weaker than that described above may be determined by ascertaining, through repeated trials, how much of solution *B* made up to 100 cubic centimeters with solution *A* will be required exactly to dissolve 10 grammes of coagulated and disintegrated albumin under the conditions given above.

In recent years the composition of the gastric juice and its variations in disease are being more and more thoroughly studied and the results of the observations made use of in clinical work. The only obstacle to the general adoption of these tests is probably the difficulty of obtaining the fluid from the stomach.

With a little experience, the collection of the gastric juice for testing can be easily accomplished. In order to excite the flow a test-meal should be given. This should be rather simple, and may consist of bread or rolls with weak tea without milk. A large amount of food rich in albuminous materials should be avoided, as the peptones resulting from its digestion interfere with some of the tests. With such a meal digestion is at its height in about an hour, and the collection should be made one to one and a half hours after eating.

The apparatus for withdrawing the juice is an elastic rubber tube about a yard in length, having a number of small perforations in the end or, if a large opening, it should be on the side, to avoid injury to the mucous membrane by suction. The perforated end is passed slowly down the œsophagus until it reaches the fundus of the stomach, known by the resistance to its further passage. The flow of the juice through the syphon is best started by pressure upon the stomach, the outer end of the tube being held lower than the stomach and over a collecting vessel. By this means the juice is not diluted.

Sometimes it is recommended to start the syphon by filling it with water by means of a funnel, then when it is full, pinching the top of the tube to close it and quickly lowering it. The first

part of the liquid which runs out is water and should be thrown away, collecting only the last part. Otherwise the amounts of constituents found must be corrected for the water, which dilutes the juice. The addition of water, however, prevents accurate quantitative tests.

Before testing the juice it should be filtered through a plaited filter, keeping it covered to avoid loss of water or acid by evaporation.

The tests which are usually made upon the gastric juice are for:—

1. Reaction.
2. Acid phosphates.
3. Hydrochloric acid.
4. Organic acids { Lactic. Butyric. Acetic.
5. Pepsin.

Others which are not so important, but the presence of which may sometimes be significant, are for:—

1. Starch.
2. Albuminous compounds.
3. Rennin.
4. Blood-coloring matters.

Quantative tests are valuable in the cases of:—

1. Total acidity.
2. Hydrochloric acid.
3. Organic acids.

The reaction of normal gastric juice is, of course, acid. In some pathological conditions it becomes neutral or alkaline. Litmus-paper can be used for the test.

To determine the total acidity of the juice filter it, keeping it covered to prevent, as much as possible, evaporation; then measure accurately 10 cubic centimeters with a pipette and place it in a beaker. Add to this a few drops of an alcoholic solution of phenolphthalein, which serves as an indicator to tell whether the liquid is acid or alkaline during the determination, being red with alkalies and colorless with acids. Then add slowly from a burette a solution containing 4 grammes of sodium hydrate to the liter, stirring continually, until the liquid is a faint pink color which remains on standing a few minutes. Enough of the standard alkali has then been added to neutralize the acid substances present. Read off this amount from the burette. If no gastric juice is at hand, a solution for experimental purposes can be made of a mixture of the above acids after greatly diluting them.

The acid phosphates may be normally present, but cannot perform the functions of the hydrochloric acid in digestion. Hence it is important to be able to detect them. They can be distinguished from free acids from the fact that they are not neutralized by calcium carbonate in the cold as the free acids are. When, therefore, a gastric juice is neutralized by adding to it finely powdered calcium carbonate, like precipitated chalk (which must itself be neutral), no acid phosphates are present, but the reaction was due to free acids. If the color of the litmus-paper is obscured by the excess of calcium carbonate, this may be rinsed off with distilled water. If the reaction remains acid after calcium carbonate has been added, acid phosphates are present. Their amount can be determined by finding the total acidity of 10 cubic centimeters, then adding calcium carbonate to 15 cubic centimeters, filtering and determining the acidity of 10 cubic centimeters of the filtrate. This

latter is due to the phosphates. The difference between the two is the amount of the free acids.

EXPERIMENT 159.—Test the reaction of the acid phosphates[1] to litmus-paper.

EXPERIMENT 160.—Show that they are not neutralized by calcium carbonate in the cold.

EXPERIMENT 161.—Show that the dilute free acids (both HCl and lactic) can be so neutralized.

For a short time after food has been taken hydrochloric acid may be wanting, or present only in traces in the gastric juice, without any pathological significance, but in one to three hours after a meal it should be found in larger amounts. It is then, under normal conditions, about 0.1 to 0.3 per cent. of the weight of the juice.

The common tests for the detection of hydrochloric acid cannot be employed in the case of the gastric juice, because the soluble chlorids which are usually present will respond to them. Special tests are used, most of which are based upon the fact that certain organic coloring matters are changed in color by a comparatively strong mineral acid, like hydrochloric, even in the dilute state, but are not affected by the weaker organic acids or acid salts. While these methods are not absolutely accurate, they are sufficiently reliable for clinical purposes when carefully performed.

Many methods have been proposed for determining the amount of hydrochloric acid. Sjöqvist's method gives accurate results. The juice is evaporated with barium carbonate, which contains no chlorids. The acids unite with

[1] Acid sodium phosphate, NaH_2PO_4, can be prepared by adding carefully orthophosphoric acid to common sodium phosphate until it does not precipitate barium chlorid. An excess of the acid must be avoided.

the carbonate, forming barium salts. The dry residue is ignited in a crucible, when the salts of the organic acids are destroyed, the barium chlorid remaining unchanged. This is dissolved with hot water and the amount determined by a standard solution of potassium dichromate. This is added until the barium is precipitated and there is a slight excess of the dichromate. The amount of dichromate used corresponds to the amount of barium present, and from this the hydrochloric acid is calculated. Toepfer's method is not difficult and the results are good.

Experiment 162.—Make some 0.2-per-cent. hydrochloric solution as in Experiment 109, and apply the following tests:—

1. Add a methyl-violet solution. The color changes from violet to blue.

2. Add a solution of tropæolin 00. The yellow color is changed to a red.

3. Add Congo red solution. The red changes to blue.

Test-paper can be made from this reagent by dipping a porous paper in the solution and drying. It can be used in testing for HCl like the solution.

4. Add to a few drops of liquid an equal volume of an alcoholic solution containing 2 per cent. phloroglucin and 1 per cent. vanillin. Evaporate to dryness in a porcelain dish on a water-bath or by carefully warming over a flame. A rose-red color remains. (Günzberg's test.)

5. To a few drops of the HCl solution in a porcelain dish add a little of an alcoholic solution of resorcin and sugar. Warm, and a red color appears. (Boas's test.)

Experiment 162*a*. Toepfer's Method for Determining the Free and Combined HCl.—Into each of three beakers (*A*, *B*, and *C*) measure with a pipette 5 cubic centimeters of gastric juice. Titrate each of them with

decinormal NaOH (4 grammes to the liter), using, as indicators, in *A* phenolphthalein, and adding the alkali until the liquid is a faint red; in *B* 3 or 4 drops of a 1-per-cent. solution of alizarin sodium sulphonate in water. The NaOH must be added until the liquid is a pure-violet color, not stopping with the reddish-violet shade. The exact shade is very nearly that given by the indicator to a 1-per-cent. solution of the common sodium phosphate. With *C* the indicator is 3 or 4 drops of a 0.5-per-cent. alcoholic solution of dimethyl-amido-azobenzol. HCl gives a red color with this, and the NaOH is added until this disappears and the color changes to yellow.

The phenolphthalein is turned red by all the compounds with an acid reaction; the alizarin sodium sulphonate reacts with all but the acid albumin; and the dimethyl-amido-azobenzol only with free HCl. The test of *A* gives the total acidity; of *C* the free, or uncombined, hydrochloric acid; and the difference between *A* and *B* the loosely-combined hydrochloric acid, or that which is in combination with the albuminous compounds. The difference between *A* and *B* — *C* represents the acidity due to the phosphates and organic acids.

Calculate from the amounts of sodium hydrate used the percentages of each of these, remembering that one cubic centimeter of the alkali contains 0.004 gramme and neutralizes 0.00364 gramme of HCl. The amounts of acid phosphates and organic acids must be expressed as equal to a certain amount of HCl.

Experiment 163. Determination of Percentage of Free Hydrochloric Acid (Sjöqvist's Method).—To 10 cubic centimeters of filtered gastric juice in a porcelain, nickel, or—better—a platinum crucible add enough pure barium carbonate to neutralize the free acid. Evaporate with a small flame to dry-

ness; cautiously burn the residue and heat it to a low, red heat. After cooling add 10 cubic centimeters of water, pulverize the residue, and pour off through a filter the water containing the dissolved barium chlorid. Repeat with successive portions of water until the filtrates together amount to about 50 cubic centimeters. Determine in this filtrate the amount of barium chlorid formed from the hydrochloric acid in the following manner: Make a solution of 10-per-cent. acetic acid and 10-per-cent. sodium acetate in water and add 3 to 4 cubic centimeters of this to the filtrate to be tested. Add also to the liquid one-fourth to one-third its volume of alcohol. The latter is to make the barium precipitate more completely, the former solution to prevent the presence of free hydrochloric acid. Add from a burette a solution of potassium dichromate containing 7.35 grammes per liter until it is in excess. This is shown by taking out a drop from time to time on a glass rod and putting it on a piece of tetra-methyl-para-phenylene diamin paper. An excess of the dichromate is denoted by a blue color. When a faint-blue color is produced, read from the burette the amount of the dichromate solution used. Calculate from this the amount of acid in the gastric juice. The reactions which occur are

$$2HCl + BaCO_3 = BaCl_2 + CO_2 + H_2O$$

when the acid is neutralized by the carbonate, and

$$2BaCl_2 + K_2Cr_2O_7 + H_2O = 2BaCrO_4 + 2KCl + 2HCl$$

when the barium salt is precipitated by the dichromate.

One molecule of potassium dichromate, weighing 294, precipitates two molecules of barium chlorid containing four atoms of chlorin. These four chlorin-atoms were derived from four molecules of hydrochloric acid weighing 4 x 36.4. Therefore, for every 294 parts by weight of the dichromate used, 145.6 parts by weight of hydrochloric acid were present. One cubic centimeter of the dichromate solution contains 0.00735 gramme, and is, therefore, equal to 0.00364 gramme of hydrochloric acid.

The weight in grammes of the hydrochloric acid present in the gastric juice in the free state or combined with the albuminous compounds is, therefore, obtained by multi-

plying the number of cubic centimeters of dichromate used by 0.00364, and, taking the weight of 1 cubic centimeter of gastric juice as 1 gramme, the percentage can be calculated. If 10 cubic centimeters of juice were used, it is only necessary to multiply the weight of HCl in this by 10 to give the percentage.

Experiment 164. Volumetric Determination of the Free Acids and Acid Phosphates.—To 10 cubic centimeters of filtered gastric juice[1] add 5 cubic centimeters of concentrated calcium chlorid solution and a few drops of phenolphthalein as an indicator, then $^1/_{10}$ normal sodium hydrate until it is neutralized, when the color is a faint pink. The amount of alkali used corresponds to the total acidity of the juice. Then take 15 cubic centimeters more of the filtered gastric juice and add about a gramme of finely-powdered calcium carbonate. Stir well and filter through a dry paper. Measure 10 cubic centimeters of the filtrate into a small flask and by means of a rubber bulb or aspirator (not with the lungs) blow air through it to remove the carbon dioxid. Then add 5 cubic centimeters of the calcium chlorid solution and phenolphthalein and neutralize with standard sodium hydrate as before. Since the free acids are neutralized by the calcium carbonate, the sodium hydrate used in this second determination corresponds to the acid phosphates, and the difference between the two to the free acid.

Subtract the number of cubic centimeters of alkali used in the last determination from that used in the first. If lactic and volatile acids are present and have been determined, subtract also the number of cubic centimeters required to neutralize them. The remainder has been used to neutralize the hydrochloric acid. Calculate the percentage of the latter, remembering that 1 cubic centimeter of sodium hydrate equals 0.00364 gramme of hydrochloric acid.

Lactic acid changes a solution of gentian violet and ferric chlorid to a green or greenish yellow. None of the

[1] A mixture of HCl and phosphates may be used if juice cannot be obtained.

other normal or pathological constituents of the gastric juice appear to do the same or to interfere with the use of the above reagents in testing for the lactic acid.

Lactic acid can also be detected in the gastric juice by the yellow color which it imparts to a solution of ferric chlorid or to the amethyst solution which is produced by adding ferric chlorid to carbolic acid (phenol), although glucose or alcohol gives a similar color.

It is generally unnecessary to determine the quantity of lactic acid. If this is desired it can be done by measuring off 10 cubic centimeters of the juice, diluting to about 100 cubic centimeters and distilling off the acetic and butyric acids, which can be determined in the distillate. After the volatile acids have been removed by distillation until the liquid remaining is about 10 or 20 cubic centimeters, the lactic acid can be dissolved from the residue by shaking it six times with 100 cubic centimeters of ether, and distilling off the ether. The lactic acid remains. Dissolve in water, add a few drops of phenolphthalein as an indicator, and see how much decinormal sodium hydrate (4 grammes per liter) is necessary to neutralize it. Each cubic centimeter of the alkali corresponds to 0.009 gramme of lactic acid.

Experiment 165. Arnold's Test for Lactic Acid. —Use the two following solutions:—

No. 1. Saturated alcoholic solution of gentian-violet, 0.2 cubic centimeter.
Distilled water, 500.0 cubic centimeters.

No. 2. Solution of ferric chlorid (U. S. P.), 5 cubic centimeters.
Distilled water, 20 cubic centimeters.

In a porcelain dish place 1 cubic centimeter of No. 1 and add 1 drop of No. 2. A bluish-violet color results. To this add the filtered gastric juice. Lactic acid changes the color to a green or greenish yellow.

EXPERIMENT 166.—To about 10 cubic centimeters of a 4-per-cent. solution of carbolic acid (phenol) add a few drops of a solution of ferric chlorid. Then dilute with water until the color is amethyst or reddish-violet. Use this as a reagent for the detection of lactic acid. The color is changed to yellow by the lactic acid.

EXPERIMENT 167.—Test 0.2-per-cent. hydrochloric acid in the same way. The solution becomes colorless; that is, hydrochloric acid gives no color of its own, hence would not conceal the lactic acid if both should be present. Try it.

EXPERIMENT 168.—Test the lactic acid with a solution of ferric chlorid so dilute that it is scarcely colored. The yellow color is made stronger.

EXPERIMENT 169.—Show that glucose or alcohol will give a yellow color with the above reagents.

Butyric acid can be distinguished by its odor, which is that of rancid butter. It can be separated from its solution by shaking with ether. The acid is more readily soluble in ether than in water, and hence remains in the ether, and is perceptible when the latter evaporates. It can also be removed by distillation, as it passes off with the steam. The acetic acid is also volatile, and if present it distills with the butyric acid. If the steam is condensed the quantity of these volatile acids can be determined by the use of a standard solution of sodium hydrate. Since they both have the same significance, indicating fermentation, it is usually unnecessary to separate them.

EXPERIMENT 170.—Shake 10 cubic centimeters of dilute butyric acid in a test-tube with about 4 cubic centimeters of ether. Pour off the ether and repeat the operation. Allow the ether to evaporate, *away from lights and fires*, and notice the odor of the acid which remains.

The presence of pepsin in a solution like the gastric

juice is best detected by trying its digestive power on fibrin. Unless the liquid contains a proper amount of acid it must be acidified so as to contain about 0.2 per cent. of hydrochloric acid. The rapidity of digestion can be best perceived by coloring the fibrin dark red with a solution of carmin in ammonia. This coloring matter is insoluble in water, but is set free, coloring the liquid, as the fibrin dissolves. The depth of color denotes the amount digested. The colored fibrin may be kept on hand for any length of time by pressing out most of the water, then preserving in glycerin or ether.

Experiment 171.—Stain to a deep red some shreds of washed fibrin with a solution of carmin dissolved in ammonia. After washing with water place these in several test-tubes; add, to each, specimens of natural or artificial gastric juice, containing different amounts of pepsin, including one with none. If they are not sufficiently acid make them so. Set the tubes in a beaker of water of about body-temperature and notice the setting free of the color as the pepsin acts.

The rennin is detected by neutralizing and testing with milk for the power of coagulation as in Experiment 157.

The test for starch and its first decomposition-product, dextrin, is iodin dissolved in potassium iodid. According to von Jaksch, neither of these remains in the stomach in normal digestion after the first hour, though they may be present as long as that when there is an excess of acid or a deficiency of ptyalin in the saliva.

The albuminous substances and digestive products can be detected by the methods given in Experiments 152, 153, and 154, and by the tests described for albuminous compounds.

The gastric juice never normally contains blood, but this is sometimes found in it or in the vomited material in cases of chronic ulceration of the stomach or after poisoning by the corrosive or strongly irritant poisons. The hæmoglobin is usually decomposed and the hæmatin which results gives a dark-brown color to the juice. It is best identified by the hæmin test (Experiment 205).

It is occasionally desired to test the rapidity of absorption from the stomach. This can be accomplished by determining how long a time is necessary for potassium iodid to pass from the stomach through the circulatory system into the saliva. About 5 grains of the iodid is taken in a capsule or in water, in the latter case being careful to thoroughly rinse the mouth afterward. The saliva is collected and tested every two or three minutes. Place a little upon a paper dipped in starch-paste and dried, or add it to a few drops of starch solution. Then add a few drops of a solution of calcium hypochlorite (chlorinated lime) to set free the iodin, which colors the starch blue. It should appear in the saliva in from eight to fifteen minutes.

The clinical tests for hydrochloric acid given above are open to some objections. Many are interfered with by large amounts of albumin and peptones. If they fail in the tests of a gastric juice the biuret test should be tried, and if any of these substances are present they should be precipitated by a 10-per-cent. solution of tannic acid and, after filtering, the liquid should be again tested for hydrochloric acid. There is also a limit to the delicacy of the tests, so that very minute amounts of free acid may not be detected. These are so small when they cannot be thus detected that the condition may be considered pathological.

Methyl-violet is turned blue by about $^1/_3$ of a milligramme of acid. Tropæolin 00 is of about the same delicacy. Congo red is somewhat affected by the organic acids if they are not very dilute. The test-paper made from this has the advantage of easy portability and that it can be preserved and also that something can be judged from the imparted color of the amount of acid present. With a large percentage it becomes a blue black, and with a small amount a lighter blue. Phloroglucin and vanillin

make a sensitive reagent, it being possible to detect with it one milligramme of free acid in 10 cubic centimeters of juice.

The lactic acid test—with phenol and ferric chlorid—is also in some cases uncertain. When it fails, however, the acid is not present. Some common substances, like sugar and alcohol, give the same results as the acid. When it is suspected that this is the case, the liquid should be shaken with ether to dissolve the acid, and the ether, after separation from the water, be evaporated to dryness. Dissolve the residue in a little water and test it for lactic acid. Arnold's test is more reliable.

The results obtained from chemical testing of gastric juice or vomited material are often of great aid in diagnosis. The presence of the organic acids—lactic, butyric, or acetic —more than thirty minutes after taking food indicates fermentative action, usually due to a deficiency of hydrochloric acid. After the same time a failure or excessive amount of hydrochloric acid can be considered pathological. It may be absent in acute or chronic dyspepsia and in chlorosis. It usually is not found, or is present in only small amount, in carcinoma of the stomach. With dilatation of the stomach caused by stenosis of the pylorus there if often an hyperacidity, more than 0.4 per cent. of the acid being present.

THE PANCREATIC JUICE.

The fluid secreted by the pancreatic gland contains three ferments which aid in the digestion of the food: trypsin, which decomposes the nitrogenous constituents; steapsin, which acts upon the fats; and amylopsin, which converts starch into glucose. The ferments occur in the gland in the form of inactive zymogens, but are changed to the active form a few hours after death or by the action of water or acids. The reaction of the juice is alkaline from

the presence of sodium carbonate. The extract made from the gland by means of warm water may be acid in reaction from the presence of sarco-lactic acid.

The trypsin dissolves fibrin and other albuminous substances, but differs from pepsin in that it acts in a neutral or weakly alkaline medium. With trypsin the fibrin does not swell and become transparent before dissolving, as is the case with pepsin, nor is acid albumin formed as the first stage of digestion. The principal decomposition-products of fibrin by the action of trypsin are, first, a globulin[1]; then albumose and peptones; then leucin, tyrosin, and aspartic acid. A substance called tryptophan, which gives a reddish-violet precipitate with bromine-water or chlorine, is also produced. Little else is known of it. The antipeptones are not affected by the trypsin. The hemipeptones are decomposed.

The substances produced from albuminous substances by the action of trypsin are:—

1. Globulin.
2. Albumose.
3. Peptones (called amphopeptones).
4. Antipeptone (not further changed).
5. Hemipeptone.

Leucin, tyrosin, aspartic acid, tryptophan, etc.

Leucin (amido-caproic acid),

$$CH_3(CH_2)_3CH(NH_2)COOH,$$

[1] Some albuminous substances do not give a globulin when acted upon by trypsin; *e.g.*, serum-albumin.

and tyrosin (para-oxy-phenyl-alpha-amido-propionic acid),

$$C_6H_4OHCH_2CH(NH_2)COOH,$$

are formed in the decomposition of protein substances by putrefaction as well as in the normal processes of digestion, and hence they may be found as a result of pathological processes where there is a destruction of the proteins. Leucin crystallizes in the form of thin plates, which are usually, when impure, grouped together into round knobs or balls. These can be recognized by the aid of the microscope. (Plate II, 12, *b*.) In the impure state tyrosin forms aggregations of crystals which resemble those of leucin, but when purified it appears as fine silky needles often gathered into sheaves or balls. (Plate II, 12, *c*.) Tyrosin requires for its solution 2454 parts of water at 20°. Leucin can be dissolved in 27 parts of cold water. This affords a means of separating them.

Experiment 172.—Try the digestive power of trypsin on fibrin. Prepare an artificial pancreatic juice by extracting a finely-chopped pancreatic gland of a hog or ox with lukewarm water. It is better to wait an hour or two after killing the animal, in order to allow of the formation of the active ferment. Filter the extract through cloth and add to the filtrate a little chloroform or thymol to prevent putrefaction, which easily occurs without an antiseptic. Place in the liquid some fibrin, make slightly alkaline with sodium carbonate, and allow it to stand at about body-temperature until it has all dissolved. The products obtained will depend upon the time. If the digestion is stopped too soon they will be largely albumoses and peptones, but later there will be more of the leucin and tyrosin. The best results will be obtained by digesting for several hours, or as much as a day.

Filter and add to the filtrate acetic acid drop by drop until the reaction is barely acid. The globulin is precipitated. This may be coagulated by boiling and removed by filtration, then the filtrate tested for the other products.

To a small portion add an equal volume of a saturated salt solution, then acidify with acetic acid. If the digestion had not proceeded too far there is a precipitate of albumose (deuteroalbumose). This can be tested by the albumose tests.

Saturate another small portion of the solution with ammonium sulphate after acidifying slightly with sulphuric acid. Filter out the albumoses and try the biuret test. A reddish-pink color indicates that peptones are present.

In another portion of the fluid which has digested for a long time test for tryptophan by adding bromine-water drop by drop. A reddish-violet precipitate results. It is destroyed by an excess of the reagent. Tryptophan is only found after the breaking down of the peptone molecule.

If this is present concentrate the remainder of the solution to a small bulk and precipitate the albuminous compounds from it by the addition of about twice the volume of alcohol. Filter these off and evaporate the alcohol, or distill it if there is a large quantity. Concentrate the liquid on the water-bath to a thin syrup, then let it stand until the tyrosin crystallizes out. Examine the form of the crystals under the microscope. If there is enough of the liquid to filter, remove the tyrosin and let the leucin crystallize from the filtrate. If there is only a small amount they can both be identified with the microscope. If there is a sufficient quantity they may be tested by the tests given in Experiments 178-181.

Steapsin, the second ferment of the pancreatic juice, splits the fats into glycerin and the fatty acid, with which

it was united. In the process of digestion, as it goes on in the animal body, only a small part of the fat is thus decomposed. The acid which has been set free in this manner unites with the carbonate of sodium which is present in the intestine, forming the sodium salt (a soap), and this serves to emulsify the rest of the fats by surrounding the globules with such a coating that they are not able to unite into a large mass. The sodium carbonate is not able to decompose the fat-molecule or to form a soap with the acid until the latter has been set free by the ferment, nor will it emulsify the fat if there is no fatty acid present, but a fine emulsion is produced after the decomposition, and the rest of the fat becomes thereby capable of being absorbed through the walls of the intestine.

The decomposition of the fat-molecule is as follows:—

$$\underset{\text{stearin}}{C_3H_5(C_{18}H_{35}O_2)_3} + 3H_2O = \underset{\text{stearic acid}}{3C_{17}H_{35}CO_2H} + \underset{\text{glycerin}}{C_3H_5(OH)_3}.$$

The fat-splitting ferment is easily destroyed by acids; hence it may not be found in a gland which has been kept until it has an acid reaction.

Experiment 173.—Make a watery infusion of the pancreatic gland, as in the trypsin digestion. If it is not already neutral make it so. Add to it in a test-tube a few drops of some neutral fat, like olive-oil, and let it stand for half an hour in a beaker of water at about 38°, shaking occasionally to keep the two liquids well mixed. Then test it with a piece of blue litmus-paper. It will turn the paper red from the fatty acid which has been set free.

Experiment 174.—Shake, in a test-tube of water, to which a few drops of sodium carbonate have been added, a few drops of olive-oil which does not contain free acids. (Since the oil easily becomes decomposed on standing, it may be necessary to remove

the free acids by first shaking with very dilute sodium hydrate solution and ether. Separate the ether from the water, wash well by shaking it a number of times with pure water, and allow it to evaporate at a gentle heat away from a fire or lamp. The alkali has united with the free acid to form a soap, and this has been washed out by the water, leaving the fat neutral.) If the oil is neutral it will form no emulsion with the carbonate. Add now a drop of a fatty acid like oleic acid and shake. A fine emulsion is formed immediately.

EXPERIMENT 175.—If a bottle of rancid oil is at hand, shake it with a weak solution of sodium carbonate, and notice that it contains enough of the free acids to form an emulsion at once.

The third ferment of the pancreas, amylopsin, converts starch into glucose as the ptyalin does, except that its action is more energetic. Thus it acts upon raw starch, which the ptyalin will not do, or at most only slowly.

EXPERIMENT 176.—Treat boiled and unboiled starch with a small amount of the watery solution of the pancreatic ferments made as before. Test for glucose by Trommer's test. Notice that it is found with the boiled starch in a few seconds; after a longer time with the unboiled.

EXPERIMENT 177.—To prepare leucin and tyrosin in larger quantities, take of white horn-shavings 2 parts and boil for twenty-four hours with 13 parts water and 5 parts of concentrated sulphuric acid, adding water as it evaporates. Dilute with water, and while warm neutralize with milk of lime. Filter, boil the precipitate several times with water, and filter in order to remove all the leucin and tyrosin. Unite these filtrates, and, after concentrating them by boiling, precipitate the calcium by the addition of oxalic acid without using an excess. Filter, extract the precipitate with boiling water, unite the filtrates, and evaporate until it becomes a thin syrup. The last of the evaporation should be performed on a water-bath, to avoid burning. Allow it to stand and crystallize. Tyrosin at first crystallizes out, mixed with a little leucin. Filter, concentrate the filtrate, and allow the leucin to crystallize.

Another better way to separate the mixed substances is to

dissolve them in a large quantity of boiling water to which ammonia has been added. To the boiling solution add basic lead acetate solution until the precipitate formed is nearly white. Filter, heat the filtrate to boiling, neutralize with sulphuric acid, and filter while hot. After cooling, the tyrosin crystallizes, and can be filtered off, while the leucin remains in solution. Remove the lead from the filtrate which contains the tyrosin by passing hydrogen sulphid through it, filter, and concentrate the filtrate, then boil it with freshly-prepared copper oxyhydrate. (This is made by precipitating a solution of copper sulphate with sodium hydrate and washing by decantation until it no longer has an alkaline reaction.) Part of the leucin is thrown down as the copper salt and a part remains in solution. The precipitate can be removed by filtration and the leucin obtained in the pure state by removing the copper by hydrogen sulphid and allowing the leucin to crystallize after concentration. From the deep-blue filtrate the copper salt can be obtained by evaporating it to a small volume and letting it crystallize. It forms sky-blue aggregations of crystals. If desired, the copper can be removed from these also by hydrogen sulphid. The leucin thus obtained is not as pure as the first portion.

Experiment 178.—Test the tyrosin with Millon's reagent. It gives a red color, showing the presence in the molecule of the group $-C_6H_4OH$.

Experiment 179.—To a portion of tyrosin crystals as large as half a pea add, in a porcelain dish, a few drops of concentrated sulphuric acid and warm on the water-bath. This forms tyrosin sulphuric acid. This is diluted with water and enough barium carbonate added to neutralize it, then filtered. The filtrate contains the tyrosin compound, which, with a very dilute solution of ferric chlorid, gives a deep-violet solution of tyrosin ferric sulphate.

Experiment 180.—Evaporate a small portion of the leucin crystals with a drop of nitric acid upon platinum foil. The residue is colorless, but on adding a drop of sodium hydrate becomes yellow to brown, and on gently heating rolls around on the foil in the form of drops.

Experiment 181.—Place a few crystals of leucin in a dry test-tube and heat gently. They melt with the odor of amylamin

and sublime, the leucin appearing on the sides of the tube as wooly flakes. This may not succeed if the leucin is very impure.

THE BLOOD.

In the examination of the blood it is convenient to consider it as composed of two parts: the corpuscles and the albuminous liquid in which they are suspended,—the plasma. The plasma, on standing, separates into two parts by coagulation, the clot—or fibrin—and a liquid,—the serum.

The reaction of the blood is alkaline from the presence of the carbonate and phosphate of sodium. The specific gravity varies from 1.045 to 1.075, with an average for adult human beings of about 1.055.

The color of the blood is caused by the red corpuscles. Even comparatively thin layers of the blood are opaque from their presence. The coloring matter (hæmoglobin) can be set free from the inside of the corpuscle by water or by many chemical reagents. The color becomes then much darker, since the light is no longer reflected from the surface of the corpuscles. The addition of strong neutral salt solutions to blood turns it bright red, because of the increased reflection of light from the shriveled corpuscles.

The red corpuscles of the same species of animals have the same shape. The average size of those of one animal of a species will be the same as that of any other, although the size of the individual corpuscle may vary greatly in the same animal. In most mammals they are round, biconcave, non-nucleated disks. In the blood of birds, amphibians, and most fishes they are nucleated and more or less elliptical. A single corpuscle when seen under the microscope has a yellowish color, not a red. The

size of the red corpuscles can be greatly changed by adding water or strong solutions of neutral salts. When water is added it passes in by diffusion, and the corpuscle swells and may burst. Likewise by diffusion when they are placed in a liquid and it contains more salts than the blood, water passes out and the corpuscle becomes smaller and shriveled in appearance. In order to dilute the blood without changing the size a solution containing 0.5 to 0.6 per cent. of sodium chlorid can be used.

Freshly-drawn blood, when allowed to stand undisturbed, in a few minutes becomes thickened to a dark-red gelatinous mass. If the coagulation is slow the red corpuscles have time to sink and collect with the fibrin in a mass in the lower part of the vessel. The serum is squeezed out of the mass and surrounds it, above and at the sides. If the blood is beaten during the time of coagulation the fibrin does not separate as a gelatinous substance, but in stringy masses, which have a high degree of elasticity. The coagulation can be prevented or hindered by cold and by the addition of neutral salts, peptones, and some other substances.

To determine the number of red corpuscles the apparatus of Thoma-Zeiss may be employed. This consists of two pieces: a pipette for measuring and diluting the blood and a cell for counting the number with the aid of a microscope. The lower part of the pipette is a graduated capillary tube for measuring the blood. Above is a bulb which, being filled to the mark with the diluting fluid, dilutes 200 times the blood which was measured in the capillary tube. The counting-cell when covered with a cover-glass gives a layer of blood 0.1 millimeter in depth. On the bottom of the cell are ruled sixteen squares, each $^1/_{400}$ of a square millimeter in area. They are surrounded by two rows of smaller rectangles. The volume of blood over each of these squares, then, must contain $^1/_{4000}$ cubic millimeter. If the number of corpuscles which are contained in this $^1/_{4000}$ cubic millimeter is determined, the number in

any volume can be found by multiplication. Several different solutions have been proposed for the dilution of the blood, one of the most convenient being a 3-per-cent. solution of sodium chlorid. For clinical purposes the blood is best obtained from the tip of the finger. For testing the method defibrinated blood from the slaughter-house may be employed. The dilution may be made twice as great by filling the capillary only to the 0.5 mark and diluting as before. In this case the number of corpuscles in each square must be multiplied by 8000 to give the number in a cubic millimeter.

The average number of red corpuscles normally present is 5,000,000 per cubic millimeter in the case of a man, and 4,500,000 per cubic millimeter of a woman. This may vary greatly in disease.

Experiment 182. Determination of the Number of Red Blood-corpuscles.—Fill the capillary tube of the pipette with blood to the mark 1, drawing it in slowly by suction with the mouth and avoiding the presence of air-bubbles in the tube. Quickly wipe dry the end of the pipette with filter-paper and draw in the diluting salt-solution (3 per cent.) to the mark 101. Close the lower end of the pipette with the finger, then compress the rubber tube at the upper end of the pipette and shake to thoroughly mix the fluids. The small glass bead in the bulb aids in this mixing. Allow the salt solution to flow out of the capillary, place a drop of the diluted blood on the ruled side and cover it with a cover-glass so that air-bubbles are not inclosed. When the corpuscles have come to rest, count the number in all sixteen squares. Count those upon the upper and left hand line of each square as belonging to the square. Use an objective which will magnify 100 to 200 diameters. The squares should be taken in some definite order to avoid counting the corpuscles in the same one more than once. The average number of corpuscles multiplied by 4000 gives the number in a cubic millimetre of the diluted blood. Multiply this by 100 for the original blood. Since a slight error in the average of each square would make a considerable error when multiplied by 4000, it is advisable to repeat the filling of the cell several times before taking the average. After using, the pipette should be rinsed out first with the diluting salt solution, then successively with water, alcohol, and ether, and finally dried by blowing dry air through it.

Experiment 183.—Separation of the Corpuscles from the Serum.—Add 30 cubic centimeters of a saturated solution of sodium chlorid to 270 cubic centimeters of water, then mix with it 30 cubic centimeters of blood, which has been defibrinated by beating it while freshly drawn. Pour it into a flat-bottomed, shallow dish and allow it to stand until the corpuscles have settled. Decant the serum, and, after mixing the corpuscles with more salt solution as before, allow to settle and decant again. By this means the serum can be entirely removed.

Directions for obtaining the serum-albumin and serum-globulin have already been given (Experiment 73).

Experiment 184. Determination of the Specific Gravity of Blood.—Prepare a mixture of benzol and chloroform, of which the specific gravity when tested with a sensitive hydrometer shall be somewhat less than that of blood. Into this mixture allow a drop of blood to fall. Freshly-drawn blood—for example, from the end of the finger—is best, though the method can be demonstrated by the use of defibrinated blood. When the blood has sunk, add chloroform, drop by drop, stirring meanwhile, until the drop floats in the midst of the liquid; that is, it has the same specific gravity. Then filter out the blood, covering the funnel to prevent evaporation, and determine the specific gravity of the mixture by means of a sensitive hydrometer. The mixed liquids can be preserved for future tests.

Experiment 185.—Test the reaction of fresh blood, using a piece of neutral, glazed litmus-paper. After rinsing off the blood with a little distilled water the paper is blue.

Experiment 186.—To a little blood in a test-tube

add an equal volume of water, and notice the change in color.

EXPERIMENT 187.—To another portion add as much saturated salt solution and observe that the color becomes brighter.

EXPERIMENT 188.—Examine each of these under a microscope and compare the appearance of the corpuscles with those of the fresh blood.

EXPERIMENT 189.—Place in a test-tube half an inch of a cold, saturated solution of sodium sulphate. Open the carotid vein of a rabbit or other small animal and allow twice as much blood to flow into the tube. Collect as much more in a clean, perfectly dry tube and allow both to stand twenty-four hours. In the first tube there is no coagulation, but the corpuscles settle toward the bottom. In the second the corpuscles are mostly held in the mass of coagulated fibrin from which drops of serum are pressed out.

EXPERIMENT 190.—Separate the serum from blood by collecting the freshly-drawn blood in a shallow vessel and letting it stand covered until it has coagulated and the serum is pressed out by the contraction of the coagulated mass.

EXPERIMENT 191.—Show that the serum contains albumins by testing a diluted solution by

1. Heat.
2. Biuret test.
3. Xanthoproteic test.

EXPERIMENT 192.—Prepare fibrin by beating freshly-drawn blood with a fork or a bundle of switches. When it has coagulated, pour off the liquid and preserve it for further tests. Wash the fibrin, at first in water to which a little salt has been added, then in clear water. Break up the large clots and continue the washing until the coloring matter is removed. If it is desired to keep it, it can

be preserved in a 1-per-cent. solution of corrosive sublimate.

EXPERIMENT 193.—After noticing the structure and elasticity of fibrin apply the following tests:—

1. Xanthoproteic.
2. Insolubility in hot and cold water.
3. Swelling and gradual solution in dilute acid.

EXPERIMENT 194.—Dissolve a little dried blood in nitric acid with the aid of heat. Filter and test the filtrate for iron with potassium ferrocyanid, which produces a blue color.

HÆMOGLOBIN AND ITS DERIVATIVES.

I. HÆMOGLOBIN: Composed of an albuminous substance and an iron compound,—hæmochromogen.

II. OXYHÆMOGLOBIN: A compound of oxygen with hæmoglobin.

III. METHÆMOGLOBIN: Composition same as oxyhæmoglobin. Different arrangement of the atoms.

IV. HÆMATIN: The iron compound united with albuminous substance in oxyhæmoglobin. Hæmochromogen plus oxygen.

V. HÆMIN: A compound of hæmatin with HCl, one molecule of each.

VI. HÆMOCHROMOGEN: Hæmoglobin minus its albuminous constituent. With oxygen it gives hæmatin.

VII. HÆMATOPORPHYRIN: Formed by the removal of iron from hæmatin, hæmin, etc.

HÆMOGLOBIN.

Hæmoglobin, sometimes called reduced hæmoglobin, is the coloring matter of venous blood. It contains iron,

besides the elements which enter into the composition of albuminous substances. The constitution of the molecule has not yet been determined, although the formulæ of some varieties are known approximately; but it is very complex, containing a large number of atoms. Like the other members of the proteid class, it contains an albuminous substance, united in this case with an iron compound. It easily unites with oxygen from the air, taking up one molecule of oxygen for each molecule of hæmoglobin and forming the readily-decomposable compound, oxyhæmoglobin. It also forms compounds with carbon monoxid (CO), nitric oxid (NO), and sulphur, all of which are similar to its oxygen compound.

Hæmoglobin can be obtained from oxyhæmoglobin by the removal of the oxygen. This may be effected either by a vacuum, by driving it out by means of a gas which itself does not act on the blood, or by the use of some chemical reducing agent. It is obtained in the crystalline state with more difficulty than its oxygen compound. It is soluble in water, giving a reddish-purple solution.

The spectrum of hæmoglobin is of great value in testing for its presence, and the same might be said in the case of the hæmoglobin derivatives. When a dilute solution of blood is held before the slit of a spectroscope, the tube being turned toward a window, the solar spectrum, consisting of the seven primary colors crossed by fine dark lines, is seen, and in addition one or more dark bands, which are due to the coloring matters of the blood. That of hæmoglobin has one broad band with rather indistinct edges lying between the *D* and *E* lines of the solar spectrum. If the liquid in the tube be shaken with air oxyhæmoglobin is formed, which has two dark bands. For clinical purposes the direct-vision or pocket spectroscope will probably be

found to be the most convenient form of instrument. (Figs. 1 and 2 on Plate IV show the spectra.)

A fresh alcoholic solution of guaiacum when oxidized gives a blue color. Many oxidizing agents, like hydrogen peroxid and oil of turpentine which has absorbed oxygen by standing for some time exposed to the air, will not act on the guaiacum alone, but will do so if hæmoglobin or its compounds are present to serve as a carrier of oxygen. This is often used as a test for blood, but is only useful in a negative way, for protoplasm will give the same reaction. It can consequently be obtained from any cell, like pus or mucus, or even from such substances as the potato. If the reaction fails, however, there can be no blood present.

Since hæmoglobin is a proteid, containing an albuminous substance, it will give the general reactions of these compounds.

The determination of the amount of hæmoglobin in the blood is made by comparing the color of the diluted blood with a solution containing a known weight of hæmoglobin, or with some other colored liquid or solid. For making a standard solution of hæmoglobin the recrystallized substance is used (Experiment 208). A solution of this can be preserved in a corked bottle or sealed tube for an indefinite time. The strength is ascertained by evaporating to dryness a given volume, and weighing the residue of hæmoglobin. This method gives accurate results.

Experiment 195. Determination of the Amount of Hæmoglobin in Blood.—Dilute a solution containing a known weight of hæmoglobin with distilled water until it is a very light-red color. Dilute in the same way the blood to be tested until the color is the same. For comparison of colors the two solutions may be placed in flat-bottomed tubes of colorless glass (Nessler tubes) or in small, flat, glass boxes, the breadth of which between the parallel sides is not more than a centimeter. Reckon from

the amount of dilution the amount of hæmoglobin compared with the standard solution, also the absolute weight and percentage.

For clinical purposes a convenient instrument for the determination of the amount of hæmoglobin in the blood is that of *Fleischl*, called an hæmometer. It consists of a short, vertical cylinder for holding the blood, separated by a partition into two compartments; a long movable wedge of ruby glass under one compartment for a standard of color, and a white surface below for reflecting the light up through the wedge and cylinder to the eye of the observer. The amount of hæmoglobin is found by filling one compartment of the cylinder with diluted blood, and the other, over the ruby prism, with water. The prism is then moved until the depth of color is exactly the same as that of the blood, when the percentage of hæmoglobin compared with the normal amount can be read from the scale. The following is the process:—

Use for a light a lamp or yellow gas-flame, not an incandescent light or daylight. Any blood may be used for practice. In clinical cases use that obtained from the tip of the finger by the aid of a lancet. After making the incision force out a drop of blood by gentle pressure. Measure off the required volume of blood (6 ½ cubic millimeters) by filling the small glass tube, open at both ends and mounted on a handle, which accompanies the hæmometer. This is accomplished by holding it horizontally and dipping one end into the drop. Wipe carefully all blood from the surface of the tube. This will be more readily done if the surface of the tube is kept slightly greasy by being preserved in an oily piece of chamois. The blood must exactly fill the tube, having neither a convex nor a concave surface at the open ends.

The compartment over the ruby-glass prism is to be filled with distilled water by means of a pipette and the other one not more than a quarter full. Into the latter the open glass measuring tube is dipped before the blood has coagulated, and the hæmoglobin is dissolved by moving the tube back and forth so as to wash out the blood. Then rinse off the tube into the blood solution by the use of a few drops of water from the pipette. Fill the compartment from a half to three-fourths full of water and stir well with a wire or with the handle of the measuring tube. Mix the water

with the blood until no turbidity is seen and until it is evident that the fluid in the angles is completely incorporated with the rest. Now drop water from the pipette upon the blood solution until it, as well as the water in the other compartment, comes exactly to the top of the division between the compartments. If the tip of the pipette is placed slightly below the surface and the water flows slowly it will not mix with the solution of blood below. This is advisable in order to prevent the possibility of any of the hæmoglobin's passing over into the other compartment. If both compartments are filled to the top of the separating partition, so that there is no meniscus at the top of the liquid, they appear, when looked at from above, to be separated only by a narrow black line. A little grease on the top of the partition will help to prevent a mixing of the two liquids. (Some authors recommend that the water be allowed to rise above the partition and a cover glass be then laid on top to prevent currents.)

Place the instrument with the large screw at the right, turn the reflector so as to illuminate the solution, shade the eye from other light, and move the ruby prism so that the shades of red in the two compartments are the same. The figure in the scale, opposite the middle of the cylinder, gives the percentage of hæmoglobin as compared with the average amount found in normal human blood.

OXYHÆMOGLOBIN.

The crystalline form of oxyhæmoglobin differs when its source is from different species of animal: from human blood being in long prisms; from the squirrel flat, six-sided plates; and from the guinea-pig, tetrahedral. It can be easily crystallized from the blood of the dog, guinea-pig, and rat, but with more difficulty from human blood or ox-blood. The color of the crystals is a bright red, and their solution is a much brighter red than that of hæmoglobin, which, when pure, approaches a black.

Oxyhæmoglobin is formed by the union of a molecule of oxygen with one of hæmoglobin, and it can without difficulty be changed back into hæmoglobin. The oxygen

is in this compound very loosely united. Oxyhæmoglobin may be also considered as composed of an iron compound, hæmatin, with an albuminous substance. It is decomposed into these two substances when its solution is heated, this change being hastened by acids or alkalies. When heated with glacial acetic acid and a little sodium chlorid it is decomposed, the hæmatin uniting with the nascent HCl formed at the same time and giving hæmin, the microscopic crystals of which have a brown color and characteristic form. This is one of the best proofs for the presence of blood, although it does not distinguish between the different kinds. No other known substance gives crystals of this color and shape.

The spectrum of oxyhæmoglobin consists of two dark bands: a narrow one at the right of the *D* line in the yellow and a broader and less distinct one in the green at the left of the *E* line of the solar spectrum. They can be made to vary in width as well as distinctness by making the solution more or less dilute. They can be perceived when it contains 1 gramme of oxyhæmoglobin in 10 liters of water; that is, 1 part in 10,000. If to this solution is added a few drops of ammonium sulphid, which has a reducing action, the oxygen is removed and in a few minutes the one broad band of hæmoglobin is seen in that part of the spectrum between the two oxyhæmoglobin lines. It is not so distinct as are the two lines and the solution may have to be strengthened to make it plainly visible. The two lines reappear upon shaking the solution with air, the oxyhæmoglobin being formed again.

METHÆMOGLOBIN.

In its percentage composition methæmoglobin differs very little, if any, from oxyhæmoglobin, and probably is

formed by a rearrangement of the atoms in the oxyhæmoglobin molecule. It is produced whenever oxyhæmoglobin is dried in the air at ordinary temperatures or when it is acted upon by weak acids. Certain oxidizing agents also will convert oxyhæmoglobin into methæmoglobin. It is also found in transudations and cystic fluids which contain blood; moreover in the urine during hæmaturia and hæmoglobinuria, as well as in the blood itself in certain cases of poisoning or after a large destruction of blood-corpuscles by burns of the skin.

In the methæmoglobin molecule the oxygen is more firmly attached than in oxyhæmoglobin, being removable neither by a vacuum nor by another gas. Like oxyhæmoglobin, however, it is changed by weak acids or alkalies into hæmatin and an albuminous substance. Like oxyhæmoglobin, too, it is converted by reducing agents or by putrefaction, where reducing forces are at work, back into hæmoglobin.

Methæmoglobin crystallizes in brownish-red needles or sometimes in plates. It is easily soluble in water, giving a brown solution, which becomes red on the addition of an alkali. The spectrum of the alkaline methæmoglobin solution has two bands much similar to those of oxyhæmoglobin, one on the *D* line, the other near the *E* line.

HÆMATIN AND HÆMIN.

Hæmatin is the iron compound which is combined with an albuminous substance to form oxyhæmoglobin. It is set free whenever oxyhæmoglobin is decomposed by the action of the gastric or pancreatic juice or by an acid. It is consequently found in the intestine after the eating of meat; also in the stomach after poisoning by a mineral acid.

The formula is given as:—

$$C_{34}H_{35}N_4FeO_5 \text{ or } C_{32}H_{32}N_4FeO_4.$$

It may be obtained from hæmin, its compound with hydrochloric acid. It is an amorphous substance, dark brown or bluish black.

Hæmin is composed of hæmatin and hydrochloric acid, probably one molecule of each. It forms microscopic crystals which, in a large amount, have a blue-black color. Under the microscope they are brown, rhombic prisms. They are sometimes separate, but two are often crossed or several are collected in clusters or rosettes. (Plate I, 3.) They are insoluble in water, but dissolve in alkalies, the hæmatin being set free. They are often called Teichmann's crystals, and are important in proving the presence of blood.

CARBONIC OXID HÆMOGLOBIN.

When carbonic oxid, either pure or mixed with other gases, is breathed or passed through blood, it unites with the hæmoglobin, forming CO-hæmoglobin: a compound similar to oxyhæmoglobin. It is, however, a more stable compound, the oxygen being unable to drive out the CO and take its place. Consequently the hæmoglobin is rendered useless as a carrier of oxygen, and cases of poisoning by coal-gas, which contains carbon monoxid, are often followed by fatal results. The compound has a two-band spectrum much like that of oxyhæmoglobin, but differs in not being changed to hæmoglobin by reducing agents. The crystals are similar to those of oxyhæmoglobin, but more of a bluish red. When mixed with strong sodium hydrate solution blood containing carbonic oxid gives a red mass, while pure blood turns brown, with a greenish cast.

HÆMOCHROMOGEN.

As the oxyhæmoglobin by the action of acids or alkalies is decomposed into an albuminous substance and hæmatin, so by the same agencies hæmoglobin gives an albuminous substance and hæmochromogen. The latter in the presence of free oxygen is converted into hæmatin. Hence, as we should expect, by the removal of oxygen from hæmatin by the aid of reducing agents we obtain hæmochromogen. The spectrum of hæmochromogen, in an alkaline solution, has two bands similar to those of oxyhæmoglobin, but a little farther toward the blue. The color of the alkaline solution is a cherry red. It is often seen in alcoholic specimens of the liver, muscles, etc., which have stood for a time in alcohol.

HÆMATOPORPHYRIN.

By the action of acids upon hæmatin the iron is removed, leaving a violet to red coloring matter—hæmatoporphyrin. It is found in the contents of the stomach and intestine after poisoning with strong acids. It also is found in some of the dark-colored urines. It is insoluble in water, more soluble in acids, and easily so in alkalies.

EXPERIMENT 196.—Add enough blood to a little water to color it a bright red. Dissolve a few crystals of ferrous sulphate and a little tartaric acid in a few cubic centimeters of water, and add a few drops to the blood solution. The oxyhæmoglobin gives up its oxygen to the iron compound, becoming changed in a short time to hæmoglobin, as is shown by the dark color.

EXPERIMENT 197.—Shake the dark solution of hæmoglobin with air and notice the change in color to a scarlet, showing the formation of oxyhæmoglobin.

EXPERIMENT 198.—Examine a very dilute solution of blood with the spectroscope in the following manner: First examine the solar spectrum by looking through the spectroscope with its slit directed toward a window. Close

the slit to a very narrow opening, and focus by sliding the focusing tube until the fine, dark lines are seen clearly. There are hundreds of these so-called Fraunhofer lines in the spectrum of the sun, but with an ordinary instrument many are indistinct. A few of the most prominent should be noticed and used to locate the position of the dark bands in the spectra of hæmoglobin and its derivatives. The most noticeable are the *C* line in the red, the *D* line in the yellow, the *E* and *b* not far apart in the green and *F* in the blue. If there are fine, black lines running lengthwise of the spectrum they are caused by dust in the slit. Next make solutions of blood and water of different dilutions and examine the spectrum which is given when a test-tubeful of the solution is held before the slit after the solution has been shaken with air to form oxyhæmoglobin. Notice the position of the two bands and observe that this does not change with the different dilutions, although the bands may be wider or more distinct when the solution is concentrated.

EXPERIMENT 199.—To the solutions of oxyhæmoglobin add a few drops of ammonium sulphid, and after they have stood a short time examine them again with the spectroscope. Notice that when the oxygen has been removed by the ammonium sulphid the spectrum has changed to that of hæmoglobin, which consists of one broad band in the space between that formerly occupied by the two.

EXPERIMENT 200.—Shake the reduced solutions with air and see that the two bands have returned again. If any ammonium sulphid remains the oxyhæmoglobin will be changed again to hæmoglobin on standing.

EXPERIMENT 201.—Through the solution of blood pass a stream of illuminating gas for a few minutes. The carbon monoxid is absorbed, forming carbonic oxid hæmo-

globin. Examine it with the spectroscope. It gives two dark bands much like those of oxyhæmoglobin, though the one next the green is not as wide as in the oxyhæmoglobin-spectrum. Try now to reduce the compound to hæmoglobin by means of ammonium sulphid. The carbonic oxid is not expelled, the two bands remaining unchanged.

Experiment 202.—Prepare an alcoholic solution of guaiacum by dissolving some of the gum taken from the middle of a lump. No blue color is produced in the solution on the addition of a small amount of old oil of turpentine, but it is produced upon the further addition of a few drops of blood.

Experiment 203.—Try the same test on the scrapings from a potato. A blue color is produced without the aid of the turpentine or any similar oxidizing agent.

Experiment 204.—Acidify slightly a solution of blood, and heat to boiling. Notice the coagulated albuminous substance and the dark-colored hæmatin coming from the decomposition of the oxyhæmoglobin.

Experiment 205.—Filter out the hæmatin or use instead a drop of fresh blood and prepare hæmin crystals from it by first drying thoroughly on a glass slide, then adding a minute amount of sodium chlorid. Cover with a cover glass; add a drop of glacial acetic acid, which will flow under the cover. Then heat over a small flame until the acid boils. After cooling examine under a microscope. The crystals sometimes are better if the acid is added and the slide heated two or three times.

Experiment 206.—To prepare a large amount of hæmin, precipitate the corpuscles from defibrinated blood by the addition of a large excess of a salt solution which contains 1 volume of a saturated salt solution in 10 to 20 volumes of water. After twenty-four hours pour off the solution and rinse the precipitated

corpuscles into a flask by the aid of a small amount of water. Add half its volume of ether, shake, and after pouring off the ether which removes most of the fats allow the solution of blood-coloring matters to evaporate in flat dishes at ordinary temperatures to a syrup. Mix this with 10 to 20 volumes of glacial acetic acid and heat in a flask one or two hours on the water-bath. Then pour into a beaker, add several volumes of water, and allow to stand several days. Wash with water and remove the albuminous substances by boiling with acetic acid.

EXPERIMENT 207.—Saturate a small portion of blood with carbonic oxid by passing illuminating gas through it. Add to it twice its volume of sodium hydrate solution,—specific gravity, 1.3,—containing about 27 per cent. NaOH. Do the same with pure blood, and spread the products on a piece of porcelain. Notice that the pure blood gives a brown color, with a shade of green. The carbon monoxid blood has a bright-red color on porcelain. This is a useful test in cases of suspected poisoning by carbon monoxid.

EXPERIMENT 208.—Prepare crystals of oxyhæmoglobin by placing on a microscope-slide a drop of blood (one which crystallizes easily, like that of a dog, rat, or guinea-pig) and cover it with a drop of Canada balsam. Cover the whole with a cover-glass and examine it under the microscope. The crystals will form in a few minutes. They can also be made by mixing the drop of blood with a small drop of water on the slide and allowing it to evaporate until a dry ring has formed around it. Place a cover-glass over it and it will crystallize. It is well to examine several different species of blood if they can be obtained, such as guinea-pig, which gives crystals in the form of tetrahedra (four-sided); mouse, giving six-sided plates; cat or dog, giving four-sided needles.

EXPERIMENT 209.—A large quantity of crystallized oxyhæmoglobin can be prepared by the following method: Make a solution of salt containing 1 volume of saturated salt solution to 9 volumes of water. Add 10 volumes of this to 1 of defibrinated blood, and let it stand a day or two in shallow, flat-bottomed vessels until the corpuscles have settled. Pour off the clear liquid, rinse the corpuscles into a separatory funnel with the aid of as

small a quantity of water as possible, and add about as much ether. Shake, but not too violently, separate the solution of oxyhæmoglobin from the ether, and filter the former. Cool it to 0° and mix it with one-fourth its volume of alcohol which has been also cooled to 0°. Let the mixture stand at a temperature of —2° to —10° for several days, until the oxyhæmoglobin has crystallized. This occurs with the blood of dogs and rats almost immediately, but that of the ox crystallizes with much more difficulty. After crystallization filter off the crystals in the cold, and dry by pressing between filter-paper. The crystals may be purified by dissolving in a small amount of water, cooling and precipitating in the same manner, repeating several times. The crystals can be preserved for a standard in the determination of the quantity of oxyhæmoglobin in blood.

EXPERIMENT 210.—To a solution of blood in water add a few crystals of potassium chlorate or potassium ferricyanid and warm gently. Methæmoglobin is formed, the color of the solution changing from a red to a brown. Make it slightly alkaline and it becomes red. Examine the spectrum of the alkaline solution. It is much like that of oxyhæmoglobin, though the first band is broader and extends somewhat farther toward the red. It is reduced to hæmoglobin by ammonium sulphid, like oxyhæmoglobin.

EXPERIMENT 211.—Large amounts of crystalline methæmoglobin can be obtained by adding to a concentrated solution of oxyhæmoglobin enough of a concentrated solution of potassium ferrocyanid to give it a deep-brown color. Crystallize, as in the case of oxyhæmoglobin, by cooling to zero and adding one-fourth the volume of cold alcohol.

EXPERIMENT 212. PREPARATION OF HÆMATOPORPHYRIN. —Saturate 75 grammes of glacial acetic acid with hydrobromic acid at 10° and add to it in a 300 cubic centimeter flask 5 grammes of dry hæmin crystals. Heat thirty minutes on a water-bath until no more hydrobromic acid fumes escape, then pour into a liter of water. After standing several hours filter and to the red filtrate add sodium hydrate till the liquid is neutral. The coloring matter is precipitated and should be filtered off, washed, and

drained upon filter-paper. Digest the moist precipitate with dilute sodium hydrate on the water-bath; filter off the hydrate of iron, which separates, and allow the solution to stand until the sodium compound of hæmatoporphyrin has separated in crystalline masses. Dissolve these in water and precipitate the coloring matter by acidifying with acetic acid. Filter, wash with water, and after stirring the precipitate up to a paste with a small quantity of water dissolve by adding hydrochloric acid carefully. Evaporate the dark-red solution in a vacuum over sulphuric acid. The hydrochloric acid compound of hæmatoporphyrin crystallizes in brownish-red needles.

EXPERIMENT 213.—Examine the spectrum of the hæmatoporphyrin.

EXPERIMENT 214.—Dissolve a few hæmin crystals in water by the aid of sodium hydrate and examine the spectrum.

EXPERIMENT 215.—Convert the hæmin into hæmochromogen by removing the oxygen. Use for this purpose a solution of ferrous sulphate with tartaric acid, the whole being made alkaline with ammonia. Examine the spectrum.

EXPERIMENT 216.—If hæmin crystals are not at hand, prepare the hæmochromogen from a dilute solution of blood by first making it alkaline with sodium hydrate, then reducing the hæmatin thus formed by ammonium sulphid or ammoniacal ferrous tartrate.

TESTING SUSPECTED STAINS FOR BLOOD.

The following tests can be used, first on a stain made by drying a drop of blood on a piece of cloth, then upon unknown stains.

1. Soak a small portion of the stain in a few drops of water on a microscopic slide. If no color is imparted to the liquid, blood is not present or the hæmoglobin has been so much decomposed that only the hæmin test will show its presence.

2. If a red color was seen in the water try the spectroscopic test. By drying, both hæmoglobin and oxy-

hæmoglobin are changed into methæmoglobin, which gives a somewhat different spectrum. (Plate IV, 12.)

Ammonium sulphid reduces this to hæmoglobin and shaking with air gives the spectrum of oxyhæmoglobin (Experiments 198 and 199). If these are all obtained the presence of blood is proved.

3. To confirm the results, or if the stain is too small to obtain them, try to obtain the hæmin crystals as in Experiment 204. After applying the acid to the dried mass the latter should be broken up with a glass rod to insure thorough mixture. These hæmin crystals are produced only from the coloring matters of the blood. When in doubt as to their identity they should be compared with those obtained from known blood, remembering that different specimens of crystals may differ considerably in size.

4. If there is a sufficient amount of the blood, not too long exposed to the air, or if it is desirable to determine the species of animal, it may be soaked in a small amount of $^1/_2$-per-cent.-salt solution and the appearance and size of the corpuscles compared with those of known specimens or measured by a microscope with a micrometer eye-piece.

THE BILE.

The bile is normally a brown to greenish, viscid fluid with a bitter taste and a neutral or slightly alkaline reaction. It is a mixture of the secretions of the liver-cells with that from the mucous membrane of the passages, which latter contains a viscous substance similar to the nucleoalbumins. This is sometimes called biliary mucin, although it differs in some respects from true mucin.

The compounds which make up the larger part of the solid matters of the bile are the sodium salts of glycocholic and taurocholic acids. Besides these and the biliary mucin there are present fats, soaps, lecithin, and cholesterin, also a number of inorganic salts of the alkalies, alkaline earths, and iron. The color of the bile is due to the biliary pigments, bilirubin, biliverdin, etc.

The salts of the biliary acids in the bile of different animals vary in their proportions. In the case of carnivorous animals only the taurocholic acid is found; in the human bile, as well as that of most cattle, both are present. The biliary acids are both compounds of cholic acid, $C_{24}H_{40}O_5$. Glycocholic acid, $C_{26}H_{43}NO_6$, is composed of cholic acid united with glycocol, $CH_2NH_2CO_2H$; taurocholic acid, $C_{26}H_{45}NSO_7$, of cholic acid and taurin, $C_2H_4NH_2SO_3H$. They can be decomposed into their constituents by the caustic alkalies. With cane-sugar and sulphuric acid the biliary acids give a purple color, and this can be used as a test of their presence.

This test is an extremely-delicate one, and its failure indicates that biliary acids are absent. There are, however, other substances—like albumin, morphine, and amyl-alcohol—which give a similar color. In these cases the spectroscopic test should not be neglected. The biliary acids can be obtained pure by evaporating the solution to dryness, extracting with absolute alcohol, precipitating this solution with ether, and applying the test to the precipitate. The purple solution, when sufficiently diluted with alcohol and examined spectroscopically, gives a dark band between *D* and *E*, near to *E*, and another before *F*. These are not seen with albumin, etc.

In concentrated sulphuric acid they give a green color, showing a strong fluorescence. The sodium salts are obtainable from the bile by evaporating to dryness and, after dissolving in alcohol, precipitating with ether.

Cholesterin, $C_{26}H_{43}OH$, occurs in most of the fluids of the body, as well as in the bile, and the calculi or concretions of the gall-bladder, of which it forms the principal part. It is not common in the urine, but is a constant ingredient of the fæces. It is insoluble in water, but soluble in ether, chloroform, or hot alcohol. It crystallizes from ether in fine, silky needles; from alcohol in large plates containing a molecule of water of crystallization. (Plate 1, 4.) In large quantities it has the appearance of a mass of white plates with a pearly luster and a greasy feeling. It is distinguished from the fats by its insolubility in the caustic alkalies, even when boiling. It forms compounds with the fatty acids similar to the fats, the cholesterin taking the place of the glycerin. Lanolin, which is found in wool-fat, is an example. These are not easily decomposed by bacteria, hence can be advantageously substituted for the animal fats where decomposition is objectionable. The cholesterin as found in the animal body seems rather to be an excrementitious material than to have any function of its own.

The bile contains at least two well-characterized pigments or coloring matters: bilirubin, $C_{32}H_{36}N_4O_6$; and biliverdin, $C_{32}H_{36}N_4O_8$. The different colors of bile from a brown to a green are due to a preponderance of one or other of these. They seem to be formed from the blood-coloring matters, being found in old blood-extravasations and being increased in amount in the bile when the blood-corpuscles are destroyed, so that the coloring matters are set free in the plasma.

Bilirubin occurs in many biliary calculi, particularly in and around the nucleus. This is the best source of the pure substance. It is commonly an amorphous powder, orange red in color. It is insoluble in water, but can be

dissolved in chloroform, and crystallizes from the latter in plates and prisms. It unites with strong bases and in calculi occurs in union with calcium. By reduction hydrobilirubin, $C_{32}H_{40}N_4O_7$, is formed. This change takes place in the large intestine as a result of putrefactive action, and the color of the fæces is due principally to the hydrobilirubin. Bilirubin is acted upon by oxidizing agents, with the formation of biliverdin. This change takes place when an alkaline solution is left exposed to the air.

Biliverdin is an amorphous, green powder. It differs from bilirubin in being insoluble in chloroform, and the two can consequently be separated by this reagent.

Both of these biliary pigments when acted upon by yellow nitric acid, such as is formed by allowing the strong acid to stand in a bright light, undergo a change of color through green, blue, violet, and red to yellow.

Besides these two coloring matters a number of others have been described by different authors. Of them comparatively little is known. They appear to be derived from biliverdin and bilirubin, and it is to their formation that the play of colors is due when bile is acted upon by oxidizing agents. Some of these are:—

Biliprasin, greenish black.
Bilifuscin, brown.
Bilicyanin, blue.
Choletelin, yellow to brown.

Not infrequently there are found in the gall-bladder concretions, commonly known as gall-stones. They are sometimes nearly as large as a hen's egg, and may fill the bladder almost completely. They are soft and often worn away from rubbing against one another. If they are cut through the nucleus is generally found to be dark colored and composed of bilirubin-calcium. Around this are concentric layers, usually of cholesterin, but sometimes of the bilirubin-calcium. Calcium carbonate is also found in

the concretions, as well as others of the biliary pigments in smaller amounts.

EXPERIMENT 217. SEPARATION OF THE SALTS OF THE BILIARY ACIDS.—The contents of the gall-bladder of an ox should be mixed with washed sand and evaporated to dryness on the water-bath. Then pulverize the mass in a mortar, which operation is facilitated by the sand. Dissolve the biliary salts by strong alcohol, and filter. Evaporate this solution to a small volume on the water-bath, allow it to cool, pour it into a flask, and precipitate with an excess of ether, shaking, to mix thoroughly. After standing a few hours the precipitated mass is converted into clusters of silky crystals (sometimes called crystallized bile). These are a mixture of the sodium salts of the taurocholic and glycocholic acids. They can be purified by filtering, washing with water, dissolving in the smallest possible quantity of water, and precipitating again with ether. They crystallize then in long, thin, colorless crystals with a silky luster.

EXPERIMENT 218. PETTENKOFER'S TEST FOR THE BILIARY ACIDS.—Use the salts or the ox-bile. Mix in a porcelain dish or test-tube with a small amount of concentrated sulphuric acid, being careful not to let the temperature rise above 70° C. It must, however, be above 50°. Then add, drop by drop, a 10-per-cent. solution of cane-sugar, stirring with a glass rod. A red color appears. If too much sugar is added or the temperature is too high the sugar is decomposed by the acid, giving dark-brown products, which conceal the red color.

EXPERIMENT 219.—The same result may be obtained by adding the sugar to the liquid to be tested, acidifying with dilute sulphuric acid, and dipping into it a piece of filter-paper. Allow the paper to dry, or dry it at a mod-

erate heat, to avoid charring. When it is completely dry, the red color appears on the paper. If heated too highly it will be turned black by the acid.

EXPERIMENT 220.—Examine the spectrum of the colored liquid obtained in 218. There are two absorption-bands: one at *F*, the other between *D* and *E*, near *E*.

EXPERIMENT 221. PREPARATION OF THE FREE BILIARY ACIDS.—Dissolve in water some of the sodium salts obtained in 217. Add dilute sulphuric acid to it slowly, until a precipitate commences to form, then add a little ether. After standing in the cold until the acid has separated, filter, wash, and recrystallize from a small amount of hot water. This gives the glycocholic acid.

EXPERIMENT 222. PREPARATION OF CHOLIC ACID.—Add to ox-bile one-fifth of its weight of 30-per-cent. sodium hydrate and boil for twenty-four hours, adding more water to replace that which has evaporated. Then saturate the liquid with carbon dioxid, evaporate to dryness, and extract the mass with strong alcohol. The sodium salt of cholic acid dissolves, as well as some other sodium compounds. Dilute with water until the solution contains no more than 20 per cent. of alcohol, then precipitate with dilute barium chlorid as long as there is a precipitate. Filter and test the filtrate with barium chlorid, which must give no precipitate. Then precipitate the cholic acid from this filtrate by decomposing its sodium salt by means of hydrochloric acid. Let it stand several hours until it has become crystalline, then recrystallize from alcohol.

EXPERIMENT 223.—Test the cholic acid thus obtained with concentrated sulphuric acid, and notice that it gives a green fluorescence. Add a few drops of a cane-sugar solution and see that a red color appears, as with the undecomposed biliary acids.

EXPERIMENT 224. PREPARATION OF TAUROCHOLIC ACID.—For this purpose use dogs' bile, which contains the sodium salt of the acid. Evaporate, dissolve in alcohol, and precipitate with ether as in Experiment 217. The acid can be set free by adding dilute H_2SO_4, but is very soluble in water and difficult to obtain in the crystallized condition.

EXPERIMENT 225.—Add the solution of the taurocholic acid

acidified with H_2SO_4 to solutions of albumin or peptones and observe that they form insoluble compounds.

Experiment 226. Preparation of Taurin.—Mix ox-bile with an excess of concentrated hydrochloric acid, and evaporate the liquid to a small volume by boiling. Pour off the solution from the resinous mass of acids which have separated, and evaporate this liquid until the sodium chlorid has, for the most part, crystallized out. Filter, and mix the filtrate, after cooling, with strong alcohol. The taurin is precipitated. Filter and wash out the salt from this with a little water. Dissolve the precipitate in the smallest possible quantity of hot water. On cooling the taurin crystallizes out in four-sided prisms.

Experiment 227. Preparation of Cholesterin.—Biliary calculi or gall-stones are the best source of cholesterin. Powder the calculus, remove the bile by boiling water, then dissolve the cholesterin in boiling alcohol, and filter while hot. It separates from the filtrate on cooling. The insoluble residue, which consists largely of compounds of the biliary coloring matters, can be used for the preparation of these. The cholesterin may be further purified by dissolving it in an alcoholic solution of potassium hydrate with the aid of heat. After it separates on cooling, wash well with water on the filter, then recrystallize from a mixture of alcohol and ether.

Experiment 228.—Examine the crystals under the microscope. They are in the form of large rhombic tables or plates.

Experiment 229.—To a crystal of cholesterin in a test-tube or under the microscope add a drop of concentrated sulphuric acid, then a drop of iodin solution. The crystal becomes first violet, then blue, green, and red.

Experiment 230.—Dissolve a crystal of cholesterin in a few drops of chloroform in a test-tube, then add an equal volume of concentrated sulphuric acid. The chloro-

form solution becomes red, then cherry red, and purple. On pouring it into a dish it becomes blue, green, and finally yellow.

Experiment 231.—Evaporate on a piece of porcelain a small crystal of cholesterin with a drop of concentrated nitric acid. A yellow stain remains which, if treated while warm with ammonia, gives a red color. Too high heating prevents the reaction.

All these reactions can be employed for the identification of cholesterin.

Experiment 232. Preparation of the Biliary Pigments.—If biliary calculi are not available, bile may be used for obtaining bilirubin, employing the yellow or brown in preference to the green. Dilute it with a little water and add a small amount of lime-water, avoiding an excess. Mix by shaking. Pass through the liquid a stream of carbon dioxid to convert any excess into calcium carbonate. Filter out the bilirubin, which has been precipitated as the calcium compound, and wash it with water. Suspend the precipitate in water, decompose it with a slight excess of hydrochloric acid, and shake it immediately with a small amount of chloroform to take up the free bilirubin, otherwise it will oxidize to biliverdin. Separate the chloroform solution from the water and precipitate the bilirubin from it by alcohol.

Experiment 233.—If biliary calculi are at hand they may be used instead of the bile. Pulverize the calculi, then dissolve the cholesterin with a mixture of alcohol and ether. (The residue from the alcoholic extraction in the preparation of cholesterin may be used [Experiment 227].) After the cholesterin has been removed decompose with acid and proceed as in the preceding experiment, 232.

Experiment 234.—Convert a portion of the bilirubin

into biliverdin by dissolving in dilute sodium hydrate and letting the solution stand in an evaporating dish. When it has turned green, precipitate with an excess of hydrochloric acid, filter, and wash.

EXPERIMENT 235.—The biliverdin in an impure state can be obtained from ox-bile by precipitating the mucin with several times its volume of alcohol, then precipitating the biliverdin by barium chlorid. Filter, wash with water, and alcohol, then decompose with hydrochloric acid. The biliverdin is insoluble in the acid. To remove the fat it must be extracted with ether, then the biliverdin can be dissolved in alcohol, which, after filtering, is left to evaporate.

EXPERIMENT 236. GMELIN'S TEST.—To a solution of bilirubin in dilute alkali add slightly yellow, concentrated nitric acid, holding the tube in a slanting position and pouring slowly so that the acid flows down under the bilirubin solution. Notice the colored rings: green nearest the top, then blue, violet, red, and yellow next to the acid. The acid must not be too yellow or the pigments quickly oxidize and nothing is seen but a yellow color.

EXPERIMENT 237. HUPPERT'S TEST.—To an alkaline solution of bilirubin add lime-water to precipitate the bilirubin. Filter, wash with water, place in a test-tube half full of alcohol slightly acidified with sulphuric acid, and boil for some time. The bilirubin is oxidized to biliverdin and the alcohol becomes colored green or bluish green.

EXPERIMENT 237*a*.—To a little of the bilirubin solution in a test-tube add a dilute tincture of iodin so that it floats on top. An emerald-green ring is seen between the liquids.

BONE.

Bone contains an organic compound, collagen, and a number of inorganic or mineral substances. These latter

are the phosphates of calcium and magnesium, mainly the former; also calcium carbonate and small amounts of calcium chlorid and fluorid. The inorganic substances can be removed by acids, leaving the bone flexible. If a bone is heated the collagen is decomposed, with an evolution of ammonia, showing that the collagen is a nitrogenous compound. Then inflammable gases are set free. If the ignition is performed where free access of air is prevented, there remains a black mass known as bone-black or animal charcoal. The black color is due to carbon, which can be removed by burning in the air, leaving the mineral or inorganic constituents only. The bone-black is an extremely porous substance and has the power of absorbing from their solutions many of the vegetable coloring matters and also the alkaloids. On this account it is used for decolorizing liquids as well as for an antidote in cases of poisoning by strychnine and some other alkaloids.

Experiment 238.—Fill a dry test-tube one-third full of fragments of dry bone, fasten it horizontally by the upper end in a clamp, and heat, at first gently, then to as high a temperature as possible without softening the glass, moving the burner so as not to heat it in one spot. The organic matter is decomposed. First water is given off, then ammonia. Test this with a piece of red litmus-paper. An oily or tarry mixture distills off with inflammable gases. When the gas has been expelled the mineral matters of the bone remain mixed with carbon.

Experiment 239.—Take a piece of this and heat it in the air, holding it with the forceps or a piece of wire. The carbon burns away, leaving only the mineral matters as a brittle mass.

Experiment 240.—Dissolve the bone-ash in dilute

nitric acid. Notice that carbonates are present, as shown by the effervescence of carbon dioxid gas.

Experiment 241.—Test a portion of the solution for phosphoric acid, which is present in the phosphates, by making it strongly acid with nitric acid, then adding ammonium molybdate and warming gently. A yellow precipitate shows phosphoric acid.

Experiment 242.—Test another small portion of the solution for chlorids with silver nitrate. They give a milkiness or a white precipitate, but are present in very small quantities.

Experiment 243.—Test the remainder of the nitric acid solution for calcium and magnesium after removing the phosphoric acid in the following manner: Add a few drops of ferric chlorid to the solution in a beaker. The iron unites with the phosphoric acid, which was held by the calcium and forms ferric phosphate. Pour a few drops from the beaker into a test-tube and test by adding ammonia to see if all the phosphoric acid has united with the iron. If this is the case, the ammonia gives a yellowish precipitate. If not enough ferric chlorid was added the precipitate is white, and more of the iron solution must be added and the test repeated until all the phosphoric acid has been taken by the iron. Then to the solution add a solution of sodium carbonate until it is nearly neutral; that is, until the precipitate—which forms as the sodium carbonate strikes the liquid—dissolves slowly on stirring. Then add 1 or 2 grammes of barium carbonate to precipitate the ferric phosphate. Filter after warming. Precipitate the barium from the hot filtrate with dilute sulphuric acid and filter. Having thus removed the phosphoric acid, test the filtrate for calcium by making it alkaline with ammonia and adding ammonium oxalate as long

as a precipitate is formed. The calcium is thrown down as white calcium oxalate. Filter and to the filtrate add sodium phosphate. A white crystalline precipitate shows magnesium.

EXPERIMENT 244.—Test the absorptive power of bone-black by adding it to a light-blue solution of indigo and warming, then filtering. The coloring matter will have almost or entirely disappeared from the filtrate.

Directions have been given before (Experiment 113*a*) for the separation of the collagen from the mineral constituents of the bone.

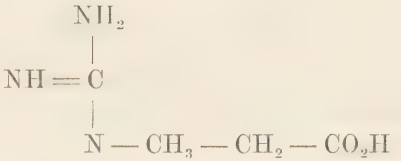
MUSCULAR TISSUE.

A muscle is made up of fibers or cells, consisting of a sheath (the sarcolemma), composed of a substance similar to elastin, and its contents. The latter are mostly albuminous matters, alkaline and liquid during life, but becoming acid and more solid after death. This albuminous liquid, which in many respects corresponds to the plasma of the blood, is called muscle-plasma. It coagulates quickly at the ordinary temperatures, and thus gives rise to the *rigor mortis* of the muscles after death. The coagulated mass is myosin: a mixture of a number of compounds. The cause of the coagulation of the myosin in this case is probably the formation of lactic acid, which accumulates in the muscle after death.

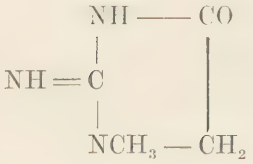
If fresh muscular tissue is treated with boiling water, most of the albuminous substances are coagulated and upon filtering remain with the fats in the insoluble residue. The solution contains, besides inorganic matters, a class of organic compounds, often called, from the method of obtaining them, the "extractives." They may be divided

into two groups: those which contain no nitrogen and those of which nitrogen is a constituent. Among the non-nitrogenous are lactic acid and its compounds; also glycogen and its derivatives: dextrin, maltose, and glucose. The principal ones of the nitrogenous extractives are creatin and creatinin, small quantities of urea and uric acid, and the nuclein bases, such as guanin, xanthin, and hypoxanthin—formerly called sarcin. Carnin, which is similar in properties and composition to the nuclein bases, is also found in the watery extract of muscle.

Creatin:



and creatinin:



are closely related, the latter being derived from the former by taking away one molecule of water, and can be changed back into creatin by adding the water again.

The nuclein bases, guanin ($C_5H_5N_5O$), hypoxanthin ($C_5H_4N_4O$), and xanthin ($C_5H_4N_4O_2$) are similar in their properties and related to uric acid ($C_5H_4N_4O_3$). They occur partly free in the muscular tissue and partly united with phosphoric acid and albuminous substances in the form of nucleins. All these soluble compounds are found,

naturally, in the various meat-extracts, which are used in foods. They are probably formed in the body by the decomposition of the albuminous materials, but their office in the vital processes is not clearly understood. When taken as a food, their value is rather in the stimulation of digestion through their agreeable taste than in their absolute nutritive worth. This may be merely because of their increasing the secretion of the digestive fluids.

EXPERIMENT 245. PREPARATION OF MUSCLE-PLASMA.—Kill a frog and immediately wash the blood from the body by passing in through a cannula inserted in the aorta a cold 0.5-per-cent. sodium chlorid solution. The necessary force can be gained by placing the solution in a doubly-tubulated bottle, which can be raised and lowered, and connecting the lower tubulure with the cannula by a small rubber tube. Cut the muscle up as quickly as possible with a cold knife or pair of scissors and freeze it by stirring in a beaker, previously surrounded by a freezing mixture of ice (3 parts) and salt (1 part). It freezes at about —7° C. Then rub it to as fine a powder as possible in a mortar, which, as well as the pestle, has been cooled below this temperature by standing in a freezing mixture. Subject the mass to a strong pressure, which gives a yellowish liquid. Filter this through muslin at a temperature below freezing. The filtrate is the muscle-plasma. Through the whole process care must be taken to preserve a low temperature to prevent coagulation.

EXPERIMENT 246.—Pour a few drops of the plasma into a dish of the ordinary temperature. It coagulates immediately.

EXPERIMENT 247.—Test the reaction of the plasma to litmus-paper. It is alkaline.

EXPERIMENT 248.—Allow the temperature of the rest of the plasma to rise slowly, and notice that it coagulates at a little above freezing. On standing a yellowish liquid is pressed out of the clot, as in the case of the coagulum of blood-plasma. This is muscle-serum.

EXPERIMENT 249.—Try the reaction of the muscle-serum to litmus-paper. It is alkaline.

EXPERIMENT 250.—Prove that the coagulated mass is a globulin (Experiments 77, 78, and 79).

EXPERIMENT 251.—Take about 500 grammes of lean beef, and, after removing, as completely as possible, the fat and connective tissue, chop it finely. Add an equal weight of water and heat half an hour on a water-bath to 55° or 60°. Filter through muslin, pressing out the water with the hands. Repeat the extraction with half as much water. Unite the filtrates, and boil to precipitate the albuminous compounds. Filter and add lead acetate as long as a precipitate forms, avoiding a great excess. Filter and remove the lead by passing hydrogen sulphid gas into the solution. Filter out the lead sulphid and evaporate the filtrate on the water-bath to 5 or 10 cubic centimeters. Allow the yellowish syrupy liquid to stand two or three days in a cool place, when the creatin crystals will separate. Filter, and wash with 88-per-cent. alcohol. Unite the filtrate and the washings and remove the alcohol by evaporation on a water-bath. After cooling make alkaline with ammonia and add an ammoniacal solution of silver chlorid. The precipitate contains the silver compounds of *hypoxanthin*, *xanthin*, and *guanin*. (The filtrate contains lactic acid. Preserve for testing.) Filter. Wash with ammonia and dissolve in boiling HNO_3, sp. gr., 1.1, to which a little pure urea has been added to prevent the decomposition of the bases. While hot, filter from a small amount of AgCl, which may remain, then allow to stand twelve hours. *Hypoxanthin-silver nitrate* separates in small needle-shaped crystals. Filter and wash with water. From the filtrate, by the addition of an excess of ammonia, is obtained a slight precipitate of *xanthin-silver oxid*. The free xanthin and hypoxanthin may be obtained by suspending their silver compounds in water and, after heating and making slightly alkaline with ammonia, adding ammonium sulphid drop by drop until the silver is precipitated, avoiding an excess. On evaporating the filtrate the xanthin and hypoxanthin will be left as microscopic crystals.

Most of the guanin is left in the precipitate made by the ammonium sulphid. It can be dissolved by boiling with a little very dilute hydrochloric acid. Filter and precipitate it from the filtrate by making it alkaline with ammonia.

To obtain the lactic acid from the filtrate from the precipitated hypoxanthin, etc., first precipitate the silver by H_2S and filter.

Concentrate the filtrate on the water-bath until most of the ammonia has been expelled. Then cool and acidify strongly with dilute sulphuric acid. The lactic acid is thus set free and can now be separated by shaking gently with about one-fifth its volume of ether, which dissolves the lactic acid, but not the sulphuric. After shaking in a glass-stoppered funnel, allow it to stand until the ether has all risen to the top of the liquid. Then draw off the water and the ether into separate flasks. Repeat the operation a few times with fresh portions of ether. Mix the different portions of ether and distill or evaporate it. The residue contains the lactic acid mixed with a little sulphuric. Dilute with water and boil a minute with zinc carbonate until it has lost its acid reaction. Filter, and evaporate the filtrate on the water-bath to a small volume. Then let it stand, and the zinc lactate will crystallize in four-sided prisms: $(C_3H_5O_3)_2Zn + 2H_2O$. Filter these from the remaining liquid, and dry on filter-paper.

To obtain the free acid dissolve some of the crystals in water and precipitate the zinc with hydrogen sulphid gas. Filter, and evaporate the filtrate. The acid will be left as a syrupy liquid. Test it for its acid reaction and sour taste. It differs from fermentation lactic acid in that it rotates the plane of polarized light toward the right. Fermentation lactic acid does not do this.

Experiment 252.—Convert a part of the creatin into creatinin by boiling fifteen minutes with very dilute sulphuric acid. Neutralize the acid by adding powdered barium carbonate as long as it effervesces. Evaporate to dryness on a water-bath and extract the creatinin from the residue with strong alcohol. Upon evaporating, the creatinin is left in the form of crystals.

Experiment 253.—Dissolve a little of the creatinin in a small amount of water, add a solution of zinc chlorid, and allow to stand. Characteristic crystals in clusters or rosettes appear. They are a double salt of creatinin and zinc chlorid.

Experiment 254.—To a creatinin solution add a few drops of a freshly-prepared solution of sodium nitroprussid, then, drop by drop, dilute sodium hydrate. The liquid becomes ruby red, soon changing to straw color. If it is now strongly acidified with acetic acid and boiled, it becomes green, then blue.

Experiment 255.—To a solution of creatinin add a few drops

of sodium hydrate, then of picric-acid solution. A red color is obtained.

MILK.

The solids of milk are partly dissolved and partly in suspension in the liquid. Of the dissolved constituents the most important are milk-sugar, an albumin, a globulin, and some mineral salts. Among the suspended compounds are casein, fat, and calcium phosphate. The average amount of solids in normal cows' or human milk is 12 or 13 per cent. by weight. The reaction of fresh cows' or human milk is nearly neutral, or may be *amphotere* to litmus; that is, it turns red paper blue and blue paper red.

The specific gravity should be between 1.029 and 1.033 at 15°, and of milk which has been skimmed after standing twenty-four hours it should be between 1.0325 and 1.0365. Thus the removal of fat raises the specific gravity and the addition of water lowers it. The average percentage composition of milk is given by König as follows:—

Cows',—water, 87.17; proteins, 3.55; fats, 3.69; lactose, 4.88; mineral matter, 0.71.

Human,—water, 87.41; proteins, 2.29; fats, 3.78; lactose, 6.21; mineral matter, 0.31.

Casein, which is a nucleoalbumin, is not in true solution in milk, since it can be filtered out by unglazed porcelain, though not by filter-paper. It is precipitated by weak acids, as is seen when the milk becomes sour, but is not coagulated by boiling. Rennin breaks up the casein into two compounds: an albumose and an insoluble calcium compound (paracasein calcium, or cheese). Coagulated human casein is not as hard as that of cows. The difference is partly due to its chemical composition, but

largely to the fact that cows' milk contains more casein and calcium than human milk. It can be made to form a soft and spongy coagulum similar to the human by dilution or by the removal of the calcium compounds.

The fats of milk are a mixture of stearin and olein, with a small amount of the glycerids of some lower members of the fatty-acid series,—butyric, caproic, caprylic, capric, etc. The fat exists as an emulsion, a coating of albumin keeping the globules separate. They may be made to collect by dissolving this coating by a chemical agent, like sulphuric acid. Babcock's method for determining the percentage of fat in milk is based upon this principle. Here the volume of the fat is measured and this gives the relative amount in the milk. If the fat rises for twenty-four hours without such decomposition it should form a layer 10 or 15 per cent. of the depth of the milk, if the latter is normal.

The method of obtaining the milk-sugar has been given. (Experiment 33.)

Experiment 256.—Test the reaction of fresh milk to red and blue litmus-paper.

Experiment 257. — Determine the specific gravity with an accurate urinometer.

Experiment 258.—Remove the fat from milk by a centrifuge, or after standing, and determine the specific gravity again.

Experiment 259.—Try the same test after adding from 10 to 25 per cent. of water to the milk.

Experiment 260.—In a weighed porcelain or platinum crucible evaporate 10 cubic centimeters of milk to dryness on a water-bath and weigh quickly to find the amount of the total solid matter. The drying will take place much more rapidly if a

weighed quantity (about 20 grammes) of dried sand is added, but the residue cannot be used for the next experiment.

Experiment 261.—Heat the dried substance in the crucible, at first gently, then until no black remains. The residue is the mineral matter, or ash. There should not be over 1 per cent. of the weight of the milk.

Experiment 262.—Compare the action of rennin upon cows' and human milk. Try the rennin in cows' milk to which 50 per cent. of water and a few drops of ammonium oxalate have been added to remove the calcium salts.

Experiment 263.—To separate the nitrogenous constituents of milk first precipitate the casein by saturating the milk with sodium chlorid. Filter and to the filtrate add powdered magnesium sulphate as long as it dissolves, stirring meanwhile. This precipitates paraglobulin: the same compound that is found in the blood. Filter and apply the globulin tests (Experiments 78 and 79). Acidify the filtrate with a few drops of dilute acetic acid and boil. The albumin of milk—lactalbumin—is coagulated.

Experiment 264.—Examine a drop of milk under a cover-glass with the microscope.

Experiment 265.—Destroy the emulsion by adding 10 cubic centimeters of concentrated sulphuric acid to an equal volume of milk. Let it stand, and the fat rises to the top in large globules. The separation is complete in a few minutes if a centrifuge is used. The volume of the fat can be better seen by using a narrow-necked flask and, after mixing with the acid, nearly filling with warm water.

Experiment 266.—Fill a 100-cubic-centimeter graduated cylinder to the upper mark with milk and let it stand twenty-four hours. There should be 10 or 15 cubic centimeters of cream.

Experiment 267. To Determine the Percentage of Lactose in Milk.—Dilute 20 cubic centimeters of milk to 400 cubic centimeters. Drop in acetic acid slowly until it coagulates; then pass carbon dioxid gas into the liquid fifteen minutes and let it stand until it settles clear. Filter and wash; coagulate the albumin and globulin in the filtrate by boiling. Filter, wash, and use the filtrate or a part of it for the sugar-determination by Fehling's solution as in the determination of glucose (Experiment 32). For every 10 cubic centimeters of the solution which is decolorized 0.067 gramme of lactose is present.

THE URINE.

The urine is a solution which contains the final products from the chemical changes in progress in the animal body. A part of these are excreted in the expired air and from the skin, and a still smaller part through the mucous membrane of the intestine, but, if we omit the carbon dioxid from the lungs, by far the greater proportion of these final products is found in the urine. A study of its composition and variation, therefore, is often of great value in judging of changes which are going on in the body.

Among the most common inorganic constituents normally found are the chlorids, sulphates, and phosphates of sodium, potassium, calcium, and magnesium. Of the normal organic compounds there are urea, uric acid and its salts, creatinin, etc. The following, when found in more than minute amounts, may be regarded as pathological: Glucose, albuminous substances, blood, bile, pus, fat, mucin, leucin, and tyrosin. Others which are more rare will be spoken of later. All of these either are taken

as such into the body with the food or are formed in the body by chemical action. The significance of each may depend upon the amount which is present, as well as upon its mere presence or absence. In interpreting the meaning of each of the constituents of the urine its method of formation must be considered, as well as the factors which may cause this to vary.

Considerable variations are found in the composition of urine which has been collected at different times of the day. That which is passed immediately after rising may differ from that excreted an hour or two after the first meal both in the kind and amount of the dissolved solids. Sugar and albumin are more commonly excreted after a meal, and may be found then, yet not be present in the night's urine. In order to obtain a fair sample for testing, the urine should be collected for twenty-four hours and, after mixing, a part taken for analysis. In all quantitative determinations the volume for twenty-four hours must be measured, and when it has been determined how much of the substance is present in the portion tested, the amount contained in the whole day's urine should be calculated. A statement of the percentage alone has little value if the quantity of the urine is not taken into account. To avoid fermentation the vessels should be clean and the tests should be made as soon as possible.

The average volume of the urine in twenty-four hours is, for an adult, between 1200 cubic centimeters and 1800 cubic centimeters (40 and 60 ounces). This, however, is subject to great variations. It is increased by diuretics, by diseases, like diabetes and others; it is diminished in febrile diseases, in acute nephritis, in some other diseases of the kidneys, and usually before the fatal termination of a disease. Its variation gives indications of the progress of the

disease. The volume will be also affected by the amount of drink or liquid food and, in general, varies inversely with the perspiration.

From the presence of ferments, the urine begins to undergo a change after it has stood a few hours. The reaction becomes alkaline, owing to the production of ammonium carbonate from the urea, and this precipitates some of the solids, so that the liquid loses its transparency. This and other decompositions produce disagreeable odors.

The odor of normal urine is characteristic. Certain foods and medicines change this; *e.g.*, oil of turpentine gives an odor of violets. When it putrefies the odor is ammoniacal and offensive. In cystitis it is ammoniacal when passed. In suppurative diseases the odor may be putrid.

Fresh, normal urine is clear, but after standing a short time a cloud of mucus appears. Pathologically it may be cloudy with matters which settle as a sediment. They will be discussed under that subject.

The color of urine is normally some shade of yellow, varying from nearly colorless to reddish yellow. The former is true of urines containing much water, and the latter where the urine is concentrated and of high specific gravity. The latter is constant in febrile conditions and their severity can here often be judged from the color. Pathologically the urine assumes many other shades. Presence of blood gives a red or, when methæmoglobin is present, a brown. Jaundice gives a greenish cast or brownish green; melanotic cancer, almost black; typhus or cholera, sometimes blue, from indigo formed by decomposition. Some medicinal or poisonous substances change the color; thus senna or rhubarb gives a reddish or brownish color, which changes to blood red on adding an alkali.

Santonin gives a yellow; carbolic acid and salol a dark green to black; antipyrin and quinine often darken it.

The specific gravity of urine varies with the amount of water and dissolved solids. With an increase of the water it approaches 1.000, and becomes greater as the solids increase. Hence it is easy to ascertain the amount of the solids which are present. If the second and third decimal figures of the specific gravity are multiplied by 2.33 it will give very nearly the weight of dissolved substances in one thousand parts of urine (grammes per liter). Thus, urine of sp. gr. of 1.021 contains about 49 grammes in a liter.

The specific gravity varies under normal conditions from 1.002 to 1.030. It is usually between 1.015 and 1.025. If sugar is not present the variation in specific gravity is due almost entirely to that of the urea. Clinically the specific gravity of urine is determined by an hydrometer, called a urinometer, which consists of a spindle weighted so as to float in pure water at the line marked 1.000. The specific gravity is indicated by the figures on the spindle at the surface of the liquid. Urinometers should always be tested in pure water and if they are not correct the reading in the urine must be changed to correspond with the error. Since the specific gravity varies with the temperature some standard temperature must be adopted. Most instruments are graduated at 60° F. (15.6° C.). The urine must be brought to this temperature before testing or, if accuracy is desired, the reading corrected by adding 1 in the fourth decimal place for every degree Fahrenheit above 60° or subtracting 1 for each degree below 60°. In order to obtain accurate results the degrees should not be too close together on the spindle.

The importance of a knowledge of the specific gravity

is rather to detect marked changes in the urine from a series of observations than to be able to infer the presence of some abnormal constituent, like glucose, which would certainly be found by the subsequent tests. Thus, in nephritis a decrease in specific gravity without change in the volume indicates that the urea is not being excreted and that uræmia may be feared.

Experiment 268.—Test the accuracy of the urinometer in water, then take the specific gravity of urine. The cylinder must be wide enough for the urinometer to float in it without touching. Foam on the liquid should be removed by a piece of filter-paper.

Experiment 269.—Test with an accurate urinometer the difference in specific gravities of freshly-passed urine when at a temperature of from 95° to 98° F. and that at 60° F. or below.

The reaction of normal mixed human urine passed during twenty-four hours is acid. This acidity is due not to a free acid, but to acid sodium phosphate (NaH_2PO_4) with the corresponding potassium salt, and varies in degree at different times. The administration of alkaline drugs is followed by the urine's becoming less acid or even alkaline. The same effect is produced by vegetable foods. These contain the potassium salts of organic acids—citric, malic, tartaric, and others—which are oxidized to potassium carbonate in the system. A similar result is brought about a short time after a hearty meal, when hydrochloric acid is being set free from its salts in the mucous glands of the stomach. The bases which are freed at the same time remain to increase the alkalinity of the blood. Part of them pass into the urine, producing the "alkaline tide," or alkaline reaction, which is often noticed at this time. The urine of herbivorous animals is normally alkaline from

this cause. On the other hand, an acid food or one from which acids are produced during its decomposition in the body will increase the acidity. Such a one is lean meat, which contains acid potassium phosphate, and also sulphur and phosphorus compounds, which form sulphuric and phosphoric acids by oxidation. Hence the reaction of the urine may be to a considerable extent regulated by the selection of foods.

Upon standing all urine becomes alkaline by fermentation. This is produced by the action of a number of microorganisms upon the urea, resulting in the formation of ammonium carbonate:—

$$CO(NH_2)_2 + 2H_2O = (NH_4)_2CO_3.$$

If these ferments are introduced into the bladder by an unclean catheter the same action is often produced there. In chronic inflammation of the urinary tract ammonium carbonate is usually present. The latter alkalinity—from ammonium carbonate—can be distinguished from that produced by sodium and potassium salts by the litmus-paper's resuming its red color after drying, if ammonia were present, but not otherwise.

In determining the degree of acidity of the urine by the use of a standard alkaline solution, litmus cannot be used to indicate when the neutralization is complete, on account of the interference of the phosphates.

Excessive acidity of the urine causes, in time, an irritation of the urinary passages, and is favorable to the formation of uric acid concretions. Continued alkalinity makes a sediment in the urine, and tends to produce phosphatic calculi. It also produces irritation or inflammation of the mucous membrane.

Experiment 270.—Test the reaction of urine with

sensitive litmus-paper, and if alkaline determine whether it is caused by ammonium carbonate by the paper's turning red again after drying, or whether a sodium or potassium compound is the alkali by the paper's remaining blue on drying.

EXPERIMENT 271. TO DETERMINE THE ACIDITY OF URINE. —To 50 cubic centimeters in a flask add 25 cubic centimeters of $^1/_{10}$ normal sodium hydrate, and heat to boiling, then remove the flame. Thereupon add 25 cubic centimeters of barium chlorid solution of about 5 or 10 per cent. Filter through a dry filter, and take 50 cubic centimeters of the filtrate, corresponding to 25 cubic centimeters of the urine, for testing. To this add a few drops of phenolphthalein for an indicator, then from a burette, $^1/_{10}$ normal sulphuric acid until the red color is just destroyed. Subtracting the number of cubic centimeters of acid used from 12.5, the number of cubic centimeters of standard sodium hydrate in the half of the liquid used, gives the number of cubic centimeters of sodium hydrate neutralized by the acids in 25 cubic centimeters of urine.

UREA.

About 86 per cent. of the nitrogen in the urine of a healthy man has been found to be in the urea, $CO(NH_2)_2$. Under pathological conditions, however, it may vary greatly from this. The absolute weight varies between 20 and 40 grammes daily, being somewhat less for a woman than for a man. In round numbers, we can say that it is about one ounce in twenty-four hours for the adult male.

Urea crystallizes in long, colorless, rhombic prisms. It is easily soluble in alcohol and in water; hence it never forms a sediment. It forms double compounds with acids, some of which, like the nitric and oxalic acid compounds are not easily soluble, and are used in separating the urea from urine. It forms similar insoluble compounds with many salts of the heavy metals, mercury, copper, etc.

When urea is brought into contact with an hypobromite or an hypochlorite, it is decomposed into carbon dioxid, nitrogen, and water:—

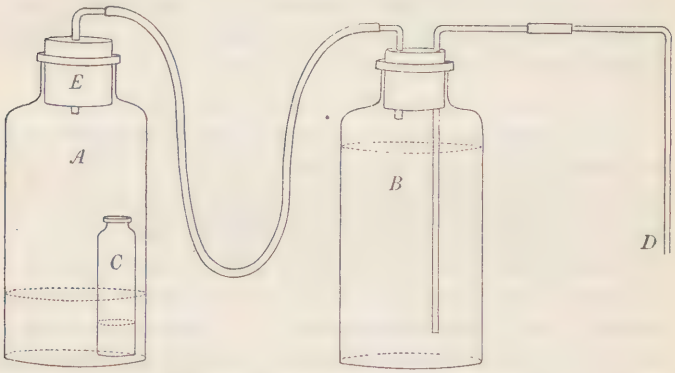
$$CO(NH)_2 + 3NaOBr = 3NaBr + CO_2 + N_2 + 2H_2O.$$

This decomposition is made use of to determine the amount of urea in urine by measuring the volume of the nitrogen set free. There are a great number of modifications in form of the apparatus employed, — Hüfner's, Doremus's, Squibb's, and many others,—all based upon the same principle. They do not give absolutely accurate results, but are sufficiently exact for clinical tests, and have the advantage of requiring but a short time for their execution. Where it is desirable to learn accurately the amount of nitrogenous compounds excreted, it is best to find the total nitrogen by Kjeldahl's method. The solution of sodium hypobromite should be freshly prepared from bromin and sodium hydrate, as it decomposes on standing.

Doremus's ureometer for determining the percentage of urea in urine consists of a short graduated tube closed at the upper end. Below it is bent upward and expands to a bulb. The graduations represent for each division 0.001 gramme of urea. That is, 0.001 gramme of urea evolves enough nitrogen to fill one division. Since one cubic centimeter of urine is used, weighing very nearly a gramme, nitrogen to fill one division corresponds very nearly to 0.1 per cent. of urea in the urine. With the tube is furnished a 1-cubic-centimeter dropping pipette.

An apparatus can be simply and cheaply made, after the principle of Squibb's, from two 4-ounce wide-mouth bottles (see figure). One of these (*A*) contains a vial (*C*), which serves to hold the urine.

Outside C, in A, is placed the solution of sodium hypobromite. B contains water and is connected with A by a rubber tube. When the rubber stoppers are tightly inserted the urine is brought into contact with the hypobromite by tipping A, the nitrogen of the urea being liberated. This forces from B an equal volume of water. The water is collected from the tube D, and when measured gives the volume of nitrogen set free. The glass tubes (D, etc.) should be of small diameter. If there is



Apparatus for Determining Urea in Urine.

no leak in the apparatus, pressing the stopper into the bottle A will force water from B into the tube D, and it should remain full, without running out, as long as the apparatus is not disturbed.

Urea is probably formed in the liver. Its source is the nitrogenous compounds of the food and the tissues, including the blood, most of the nitrogen of such compounds being excreted from the body in the urea. Hence any increase in the destruction of these substances is ac-

companied by an increased formation of urea and *vice versâ*. For this reason the urea is considered as *a measure of the decomposition of the proteins in the body.*

Some things which bring about an increased decomposition of proteins are: a large amount of nitrogenous food, like meat; excessive exercise, which causes a destruction of tissue, though here the urea is not proportional to the exertion; fevers and inflammations up to the crisis, owing to the rapid loss of muscular tissue. After the crisis it is diminished. In phosphorus poisoning and diabetes mellitus the urea is excessive for the same reason. A greater excretion of water, either from excessive drinking or diuretics, carries with it a larger amount of urea, which seems to be thus washed out of the system.

On the other hand, less urea is excreted during hunger and sleep, when the metabolism of the body is lessened. Interference with the excretory power of the kidneys likewise diminishes the urea. This is seen in acute nephritis and other diseases of the kidneys. In such cases the production of urea is not stopped, but it accumulates in the system, often being accompanied by uræmic poisoning. Since the urea is formed, at least in part, in the liver, we find that less is excreted in carcinoma and cirrhosis of this organ.

The fermentation of urea to ammonium carbonate, caused by the action of micro-organisms, has been already referred to.

PREPARATION OF UREA.

Experiment 272. From Urine.—If only a small amount is desired, evaporate half a test-tubeful of urine to dryness on the water-bath. Dissolve the urea from the residue with 95-per-cent. alcohol. Allow a drop of the

alcoholic filtrate to evaporate on a microscope-slide without the aid of heat. Examine the crystals under the microscope. If the form is not distinct, dissolve in a drop of water and again observe the crystals after this has evaporated. Add a drop of dilute nitric acid to the slide, let it stand a few minutes, then examine the crystals of urea nitrate. (Plate I, 6.)

2. A larger quantity can best be obtained by evaporating half a liter to a liter of urine to a thin syrup upon the water-bath, then cooling it in ice-water, and adding about three times its volume of nitric acid of a specific gravity of 1.3 which has been boiled to expel the oxids of nitrogen and cooled with ice-water. Filter off the urea nitrate through an asbestos or glass-wool filter, washing with a small quantity of ice-cold concentrated nitric acid. Dissolve the crystals in hot water and decolorize by chlorin-water or a small quantity of potassium chlorate. Add, then, small portions of pure barium carbonate as long as it dissolves and until the liquid is neutral. Evaporate the whole upon the water-bath to dryness. Pulverize the residue and dissolve the urea in absolute alcohol, which does not dissolve the barium nitrate. If the alcoholic solution is colored it can be decolorized by filtering it through bone-black. Distill off the alcohol or allow it to evaporate to obtain the urea.

EXPERIMENT 273. SYNTHETICALLY.—Coarsely powder 50 grammes of potassium ferrocyanid and heat in an iron dish over a Bunsen flame, stirring continually, until it has become a white powder and the lumps show no yellow color when they are broken. If it turns brown the heat is too high. Pulverize the mass as finely as possible in a mortar, mix it thoroughly with half its weight of finely-powdered manganese dioxid, and heat in an iron

dish under the hood, stirring meanwhile, until the mass glows and becomes thick and sticky. Heat until a small test dissolved in hydrochloric acid gives no blue color with ferric chlorid. Then allow it to cool; dissolve the potassium cyanate, which has thus been formed with cold water. Convert this into ammonium cyanate by the addition of 38 grammes of dry ammonium sulphate. Filter and evaporate upon the water-bath at about 60° to 70°, at which temperature ammonium cyanate is converted into urea. The potassium sulphate crystallizes out first, and should be removed from time to time. At last evaporate to dryness and dissolve out the urea with absolute alcohol as before.

Experiment 274.—Mix a few of the dry crystals with soda-lime and heat in a dry test-tube. The presence of nitrogen is shown by the evolution of ammonia.

Experiment 275. Preparation of Sodium Hypobromite.—In a thin glass flask or beaker containing 20 cubic centimeters of water dissolve 8 grammes of sodium hydrate. Cool, and from a dropping pipette or funnel add slowly 2 cubic centimeters of bromin, stirring or shaking meanwhile. Handle the bromin under a hood or in a draft of air to avoid the vapors, which are especially irritating to the eyes and lungs. As the bromin-gas is heavy, it should be held below the level of the face while pouring, rather than above.

Experiment 276.—Determine the percentage of urea in urine by the use of Doremus's ureometer. First fill the tube with the hypobromite solution and invert it, having no more of the liquid in the bulb than is necessary to keep the tube full. Fill the pipette exactly to the mark with urine, insert the lower end into the ureometer, and slowly and steadily force the urine out by compressing

the rubber bulb. The urine, being the lighter liquid, rises in the ureometer and is immediately decomposed. The carbon dioxid is dissolved in the solution and only the nitrogen is collected. No gas-bubbles should be allowed to escape into the ureometer-bulb or back into the pipette, thereby causing a loss. When the foam has disappeared, read off the quantity of gas and calculate the percentage of urea. Duplicate tests should not differ more than 0.1 per cent. If the volume of urine in twenty-four hours is known, calculate the weight of urea excreted in that time.

Experiment 277.—Determine the amount of urea in urine by apparatus of Fig. 1. Fill *B* nearly full of water. Into *C* by a pipette put exactly 2 cubic centimeters of urine. Outside of *C* in *A* put 20 or 25 cubic centimeters of the hypobromite. Insert the stopper (*E*) tightly, thus filling the tube *D* with water. If it remains full, showing that the apparatus is tight, place an empty beaker under *D* and gently mix the urine and hypobromite. Avoid as much as possible raising the temperature by holding the bottle in the hand, as the expansion of the gas causes a considerable error. Allow it to stand until no more water passes from *D*, which must remain full of water during the whole test; then measure the expelled water in a graduated cylinder.

One gramme of urea contains 371 cubic centimeters of nitrogen; but, when it is decomposed in this manner, only about 354 cubic centimeters are obtained. For ordinary clinical purposes the percentage of urea in urine can be calculated from the following formula:—

$$\text{percentage of urea} = \frac{100 \times \text{number of c. c. of N}}{2 \times 354}$$

In more accurate determinations allowance must be made for the variation in the volume of the gas from the presence of water-vapor, from the variation of the barometer, and the variation in temperature from 0° C. This formula is then to be used:—

$$W = \frac{100\ v\ (b - s)}{760 \times 354 \times u\ [1 + (0.003665 \times t)]}$$

W = weight of urea in 100 c. c. of urine.
v = volume of water = volume of N in c. c.
b = height of barometer in mm.
s = tension of water at given temperature.
u = cubic centimeters of urine used.
t = temperature in degrees centigrade.

The tension of water-vapor for different temperatures can be found in the tables given in any work on gas-analysis.

Experiment 278. Determination of the Total Nitrogen in Urine (Kjeldahl's Method[1]).—I. Prepare the following solutions:—

1. Standard sulphuric acid containing about 25 grammes per liter, of which the strength has been accurately determined.

2. Standard ammonia, of which about five volumes are necessary to neutralize one of the acid. Determine this accurately and calculate the amount of ammonia by weight in 1 cubic centimeter.

3. Potassium sulphid (commercial), about 40 grammes per liter.

4. Sodium hydrate free from ammonia and nitric acid, about 270 grammes per liter.

5. Cochineal, made by digesting for a day or two 3 grammes powdered cochineal in a mixture of 50 cubic centimeters of alcohol and 200 cubic centimeters of water, then filtering.

Have also at hand:—

1. Sulphuric acid, sp. gr. 1.84, free from compounds of nitrogen.

2. Yellow mercuric oxid.

3. Powdered potassium permanganate.

II. *Operation.*—With a pipette measure accurately 5 cubic

[1] This method can be used for finding the amount of N in most animal and vegetable compounds.

centimeters of urine. Place it in a flask holding about 250 cubic centimeters, best of hard (Bohemian) glass. Add 0.4 gramme of mercuric oxid and 10 cubic centimeters of the *concentrated* sulphuric acid. Lay the flask in a slanting position on a wire gauze over a flame small enough to just bring it to boiling. Perform this operation under a hood or where there is a good draft to carry away the fumes of the acid. Continue the heating until the liquid is colorless or straw-colored, which may require from thirty minutes to an hour. Then remove the flask from the flame and very slowly add to it a small amount of the powdered permanganate until it is colored reddish or greenish. Allow it to cool and pour it into an 800-cubic-centimeter flask which contains from 200 to 300 cubic centimeters of distilled water, rinsing the small flask into the large one. The organic matter has been oxidized in this process, the nitrogen being converted into ammonia, which is contained in solution as ammonium sulphate.

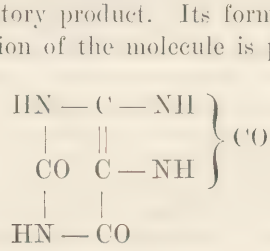
The ammonia is now to be set free by sodium hydrate, then distilled into a known amount of standard acid, and its amount found by ascertaining the loss of strength of the acid through neutralization by the ammonia. For this purpose a Liebig condenser is to be arranged so that the flask can be connected with the upper end by means of a bent tube; this should be at least $^1/_4$ inch in diameter inside and a foot long, to prevent small drops of the boiling liquid's being carried over. The insertion of a bulb between the flask and condenser, also having the lower end of the bent tube, in the flask, cut off obliquely, will aid in preventing this. Add, now, to the liquid in the flask a few fragments of granulated zinc to make it boil more quietly, 40 cubic centimeters of the potassium sulphid to precipitate the mercury, and 80 cubic centimeters of the sodium hydrate, or enough to make it alkaline. Connect immediately with the condenser through which a stream of cold water is flowing and distill into a 400 or 500 cubic centimeter conical flask (Erlenmeyer) which contains exactly 10 cubic centimeters of the *standard* acid (not the concentrated!). Continue the distillation until at least half has been distilled over and the distillate coming from the condenser no longer turns red litmus-paper blue.

Then find how much ammonia has been taken up by the standard acid. To do this add a few drops of cochineal solution to

the distillate. It will be colored yellowish, because of the acid reaction. Enough cochineal should be used to give a straw-color. From a burette add the standard ammonia, stirring meanwhile, until the straw-color just changes to a pink, when the liquid is neutral. Subtract the number of cubic centimeters of ammonia used from the number which are required to neutralize 10 cubic centimeters of the standard acid. The difference represents the volume of standard ammonia equal to that which was distilled from the oxidized urine. Calculate the weight of NH_3 in this. Fourteen-seventeenths of the NH_3 is the weight of the nitrogen in 5 cubic centimeters of urine. Calculate the percentage.

Uric Acid ("Lithic Acid").

Uric acid is normally present in solution in the urine of mammals. With birds and snakes it is the principal nitrogenous excretory product. Its formula is $C_5H_4N_4O_3$ and the composition of the molecule is probably



The daily amount varies much, but averages from 0.2 to 0.8 gramme. Except that it must be formed from the nitrogenous compounds in the body, we know little of its production or of the cause and significance of its variations.

Uric acid is comparatively insoluble in water or acids, but dissolves readily in the fixed alkalies, forming salts of uric acid, or urates. In the urine the acid exists in the form of these salts or united with some organic base. It is a dibasic acid like sulphuric acid, having two atoms of hydrogen which can be replaced by metals. It can thus

have two series of salts, the acid and the normal, corresponding to $HKSO_4$ and K_2SO_4. Of these classes the normal salts are quite soluble in water, but the acid salts do not dissolve so easily. The acid can be set free from its salts by the use of a stronger acid. The solubility of the acid salts is much less in cold water than in warm. Consequently they frequently separate from urine which was clear when passed but has stood in a cold room, and they can then be redissolved by warming.

When it is pure, uric acid exists in the form of colorless crystals. As it is found in the urine, it, as well as its salts, is always colored yellow to brown by the coloring matter which has been carried down from the urine. The simplest form of crystals is tabular with curved sides and pointed ends. These are frequently united at right angles, making a star-shaped form, two of the rays often being smaller than the other two. In urinary sediments many crystals may be united, making a rosette-like form. In strongly-acid urine the crystals sometimes have jagged edges like the teeth of a broken comb. Many different forms may be obtained by precipitating with various strengths of acid. (Plate II, 11.)

Uric acid and its salts have, in some degree, the power of reducing copper compounds in an alkaline solution and thus give with Fehling's test results which are similar to those obtained with glucose. When the dry substance is warmed with nitric acid it is oxidized, and then gives with ammonia a reddish-purple salt, which serves to detect and identify the acid.

The urates as found in the urine are either in solution or form a sediment. The latter is generally amorphous and is always colored yellow to brown. Acid sodium urate may occur in spherical aggregations of microscopic

acicular crystals. Ammonium urate, formed when urine becomes alkaline by fermentation, may be found as brownish spherules covered with irregular spicules, the so-called "thorn-apple" crystals. (Plate II, 9 and 11.) Absolutely accurate methods for determining the amount of uric acid in urine are not known. Its amount is sometimes found by precipitating from a measured volume of urine by hydrochloric acid, the albumin having first been removed if it is present. After washing the crystals they are weighed. The results thus obtained are too low, because of the slight solubility of the crystals in water. Volumetric methods may also be employed.

EXPERIMENT 279.—Prepare uric acid from urine by quite strongly acidifying a beakerful with hydrochloric acid. In twenty-four hours the uric acid will have separated. Examine the crystals under the microscope. It can be purified and gradually freed from the color which it derives from the urine by repeatedly dissolving in sodium hydrate, diluting with much water and precipitating again with HCl.

EXPERIMENT 280.—Dissolve a few of the crystals of the acid in sodium hydrate and add a few drops of Fehling's solution. Boil and the red cuprous oxid will be formed, best seen by the use of a dark background.

EXPERIMENT 281.—To a small quantity of uric acid in a porcelain dish add a few drops of dilute nitric acid and evaporate to dryness, holding the dish over a small flame with the hand in order to avoid heating too highly. A reddish-yellow residue is left. Pour into the dish a drop of ammonia without at first letting it come directly into contact with the residue. In a short time the residue becomes colored reddish purple. The ammonia may be added directly to the residue if an excess is not used. An

excess destroys the color. The addition of a drop of sodium hydrate changes the color to a bluish purple, which is destroyed on warming. The test is called the *murexid test.*

EXPERIMENT 282. PREPARATION OF CRYSTALLIZED ACID URATES.—Dissolve uric acid in not a large excess of sodium hydrate, and then pass carbon dioxid into the cold solution until it is saturated. Acid sodium urate separates in amorphous masses.

EXPERIMENT 283.—Test the solubility of the acid sodium urate by warming with a small quantity of water. It will dissolve, and, if not too much water has been used, will separate out again when it cools.

EXPERIMENT 284.—Prepare crystallized acid urates by dissolving a little uric acid in a warm solution of sodium phosphate. Filter, if necessary, and allow the filtrate to stand and evaporate. The sodium urate will crystallize as masses of acicular crystals.

EXPERIMENT 285. QUANTITATIVE DETERMINATION OF URIC ACID (HOPKIN'S METHOD).—To 100 cubic centimeters of urine add powdered ammonium chlorid as long as it dissolves, which will require about 50 grammes. Let it stand about two hours, when the uric acid will be precipitated as ammonium urate. Filter, and wash three or four times with a saturated solution of ammonium chlorid. Wash the precipitate from the paper by hot water into a small beaker, acidify with HCl, heat to boiling, cool, and allow to stand two hours or more. The free uric acid has then crystallized out. To make correction for the acid which remains dissolved, measure the filtrate before washing and add to the final result 1 milligramme for each 15 cubic centimeters. Wash the precipitated uric acid on the paper with a little cold water, then wash it off from the filter into a flask and warm it with sodium carbonate until it has dissolved. Cool and dilute to 100 cubic centimeters. Add slowly, while stirring, 20 cubic centimeters of pure concentrated sulphuric acid and titrate immediately with one-twentieth normal potassium permanganate solution. The latter contains 1.578 grammes of pure potassium permanganate in a liter; 1 cubic centimeter is decolorized by 0.00375 gramme of uric acid. The

permanganate solution should be dropped from a burette into the uric acid solution until the latter has a pink color, which remains a few minutes. The number of cubic centimeters is then read from the burette and the amount of uric acid calculated, adding the correction for solubility mentioned above.

Hippuric Acid ($C_6H_5COHNCH_2CO_2H$).

This occurs normally in the urine, but in that of human beings only in very small quantities. It is found here in larger amounts after the internal use of benzoic acid. It increases with a vegetable diet and is abundant in the urine of herbivorous animals. It forms translucent, four-sided prisms, somewhat soluble in water. The acid can be made synthetically by heating benzoic anhydrid, $(C_6H_5CO)_2O$, with glycocol, $NH_2CH_2CO_2H$:—

$$(C_6H_5CO)_2O + 2NH_2CH_2CO_2H = 2C_6H_5CONHCH_2CO_2H + H_2O.$$

When hippuric acid is heated with mineral acids or alkalies it decomposes again into glycocol and benzoic acid.

Experiment 286. Preparation of Hippuric Acid. —Make the freshly-passed urine of a horse or other herbivorous animal strongly alkaline with milk of lime. Warm, filter, and evaporate the filtrate to a syrup on the water-bath. After it has cooled acidify strongly with concentrated hydrochloric acid. Stir and filter, washing with a little very cold water. Dissolve the crystals in the smallest possible amount of boiling water. To destroy the coloring matter pass chlorin gas into the hot solution until it is light yellow. Then cool it, filter, and wash the crystals with a very little cold water. If they are still colored they can be still further purified by dissolving in water and boiling with a little animal charcoal. Filter, and let the acid crystallize from the filtrate.

Experiment 287.—Heat a few of the dry crystals in

a glass tube. They melt and turn red, then give, at first, a hay-like odor, afterward the odor of bitter almonds, from the hydrocyanic acid formed. On the cooler part of the tube is a sublimate of benzoic acid.

EXPERIMENT 288.—On a few crystals in a test-tube pour about a cubic centimeter of concentrated nitric acid, and bring to a boil. Evaporate to dryness in a porcelain dish on a water-bath. The residue, when heated in a dry glass tube, gives the odor of bitter almonds. This test can be used to detect small quantities of hippuric acid.

CHLORIDS.

In the urine the excreted chlorin, of which there is normally in a day 6 to 10 grammes, is united principally with sodium. There is a small part with potassium as potassium chlorid. The excretion of chlorids in health is increased with salt food and with large quantities of drink. Chlorids are necessary in the fluids of the body for the proper performance of their functions. When more chlorin is required by the body the chlorids are held back by the kidneys from the urine. When there is a less demand in the body the kidneys excrete the chlorids. Thus, in pneumonia and other diseases, where there are serous exudations, the chlorids are withdrawn from the circulation to form the constituents of these fluids, as is shown by their decrease in the urine. When the pathological exudations are absorbed the amount of urinary chlorids increases. In fevers there is a decrease in the chlorids of the urine until the crisis, then an increase. In chronic diseases the amount of chlorin gives some indication of the digestive power, 6 to 10 grammes per day being normal, and less than 5 grammes daily showing weakness of digestion, providing

that an excessive amount has not been removed by other means, like serous exudations or diarrhœic discharges. An excessive excretion of chlorin (15 to 20 grammes daily) is found in diabetes insipidus. In dropsical conditions it is a favorable sign, showing the absorption of the fluid.

The quantity of chlorin can be determined by ascertaining how much silver nitrate is required to precipitate it.

$$\underset{58.4\text{ parts}}{NaCl} + \underset{170\text{ parts}}{AgNO_3} = AgCl + NaNO_3.$$

To ascertain when the chlorin has all united with the silver a little yellow potassium chromate is added. The silver forms first a white silver chlorid, and when the chlorin has been precipitated it forms the red silver chromate.

Experiment 289.—Acidify a portion of urine in a test-tube with nitric acid and add a little silver nitrate. A white precipitate which turns dark in the sunlight indicates the presence of chlorids.

Experiment 290. Determination of Quantity of Chlorin in Urine.—For clinical purposes the following method is sufficiently accurate: Measure with a pipette 10 cubic centimeters of urine and dilute with about 100 cubic centimeters of water. Add a few drops of yellow potassium chromate solution; then allow to flow into it from a burette a solution which contains 17.00 grammes of fused silver nitrate in a liter. As soon as the color of the precipitate changes from white to reddish, read off the volume of silver solution which has been used. Each cubic centimeter of this will precipitate 0.00354 gramme of chlorin, equal to 0.00584 gramme of sodium chlorid. Calculate the percentage of chlorin by weight in the urine. The change of color from white to red can be more plainly seen by yellow light (gaslight) than by daylight. There

are present in the urine some other substances which are precipitated by silver nitrate like the chlorin. To make approximate correction for these, 1 cubic centimeter may be subtracted from the number used.

Phosphates.

The phosphoric acid of the urine is united with two classes of bases: the alkalies,—sodium and potassium,—and the alkaline earths,—calcium and magnesium. The compounds are called, respectively, "alkaline" and "earthy" phosphates. The alkaline phosphates are soluble in water. The earthy phosphates are insoluble in water or alkalies, but are dissolved by acids. They consequently appear in the urine in the insoluble form whenever it becomes alkaline, either by fermentation or by the addition of reagents. They may also be precipitated by boiling. The amorphous white precipitate thus obtained is often mistaken for albumin. It can be distinguished by being easily soluble in acids, which is not the case with albumin. When ammonia is present, as in fermentation, the magnesium forms an insoluble salt with two bases, NH_4MgPO_4. In urinary analysis it is referred to as triple phosphate. It is crystalline, sometimes in the form of snow-flakes, but more commonly in prismatic crystals often spoken of as "coffin-lid crystals," from their supposed resemblance to the lid of a coffin. (Plate II, 8.)

It is the acid phosphates of the alkalies (NaH_2PO_4 and KH_2PO_4) which give the acid reaction to the urine.

The phosphoric acid of the urine is mainly that taken in the food, but a part comes from the oxidized phosphorus compounds of the tissues, such as lecithin and the nuclein compounds. The presence of a sediment of the

earthy phosphates shows simply that the urine is alkaline, and is no indication that an excessive amount is being excreted. Animal foods are richer in phosphoric acid compounds than vegetable; hence with these we find more in the urine.

Experience has shown that there is a diminution of the excreted phosphoric acid in many pathological conditions. This is true in most acute infectious diseases, in nephritis, gout, and rheumatism. In diabetes mellitus there is an increase. Still, with the exception of the bones, the tissues of the body contain but comparatively small amounts of phosphorus compounds, and with our present knowledge it is difficult to draw definite conclusions regarding the decomposition of such tissues from the variations in the eliminated phosphoric acid.

Experiment 291.—Make a specimen of urine alkaline with sodium hydrate. The earthy phosphates are precipitated in an amorphous form. Examine under the microscope. See that they are dissolved again by acidifying with even a weak acid, like acetic.

Experiment 292.—Filter out the earthy phosphates and test the filtrate for the phosphoric acid of the alkaline phosphates by adding magnesia mixture. (This is magnesium sulphate made alkaline with ammonia and enough ammonium chlorid to dissolve the precipitate first formed.) With phosphoric acid it gives a white crystalline precipitate.

Experiment 293.—Form triple phosphate by making urine faintly alkaline with ammonia and allowing it to stand until the precipitate settles. Examine under the microscope for the "coffin-lid" crystals. They can be more abundantly formed for microscopic examination by adding

to the urine a little of a solution of magnesium sulphate before making it alkaline with ammonia.

EXPERIMENT 294. DETERMINATION OF AMOUNT OF PHOSPHORIC ACID.—Prepare the following solutions:—

1. Uranium acetate: Dissolve about 34 grammes of crystallized uranium acetate in water and dilute to one liter. This solution will be a little too strong. Its exact strength must be found by the method to be described later.

2. A solution of Na_2HPO_4, $12H_2O$ (crystallized disodium phosphate), one liter of which shall contain 10.085 grammes of the pure crystallized salt. This salt gives up its water of crystallization when exposed to the air, and cannot then be used. The crystals must be perfectly bright. Fifty cubic centimeters of the solution contain 0.1 gramme of P_2O_5 (phosphoric anhydrid).

3. Solution of sodium acetate of which one liter contains 100 cubic centimeters of 30-per-cent. acetic acid and 100 grammes of sodium acetate.

4. Solution of cochineal made by digesting for some time 1 gramme of powdered cochineal in a mixture of 20 volumes of alcohol with 60 volumes of water. Filter or decant the liquid.

Operation.—First ascertain the strength of the uranium solution. To accomplish this, measure with a pipette 50 cubic centimeters of the sodium phosphate solution into a beaker; add 5 cubic centimeters of the sodium acetate solution and a few drops of cochineal. Heat to boiling, and then from a burette run in the uranium solution, drop by drop, until a greenish color is produced. The phosphoric acid has then been precipitated. Since 1 cubic centimeter of the uranium solution ought to precipitate 0.005 gramme of P_2O_5, exactly 20 cubic centimeters should have been used for the 50 cubic centimeters of sodium phosphate. If this is not the quantity which has been used, first ascertain accurately how much is needed and then dilute the uranium solution so that 1 cubic centimeter precipitates 0.005 gramme of P_2O_5. If, for instance, 17.5 cubic centimeters have been used instead of 20 cubic centimeters there must be added 2.5 cubic centimeters of water for every 17.5 cubic centimeters of the uranium solution.

The amount of P_2O_5 in urine can now be determined in the same manner, using urine instead of the sodium phosphate solu-

tion. Calculate the percentage of P_2O_5 present, knowing that there is 0.005 gramme for each cubic centimeter of uranium solution which has been used.

Sulphates.

The sulphates of the urine are of two classes: (1) those of which the base is a metal, like K_2SO_4 and Na_2SO_4, and (2) those in which a part or the whole of the base has been replaced by an organic radical, like $KC_6H_5SO_4$. Those of the first class are called the inorganic, and the second the organic, or ethereal, sulphates. The latter differ from the inorganic in not forming an insoluble precipitate upon the addition of a barium salt as the inorganic do. The two classes can be separated by this means. After the removal of the inorganic sulphuric acid by barium chlorid the organic sulphates can be decomposed by means of boiling hydrochloric acid:—

$$KC_6H_5SO_4 + H_2O = C_6H_5OH + KHSO_4.$$

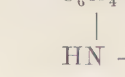
The acid will then give the white precipitate of barium sulphate if barium chlorid be added.

The total amount of combined sulphuric acid excreted by an adult in twenty-four hours is 2 to 3 grammes. It is derived partly from that already formed in the food, which passes without change into the urine, but, for the most part, from the oxidation of sulphur compounds, like albumin, in the body. Variations in the total sulphuric acid in general indicate the rate of oxidation of sulphur compounds. It is increased by taking such compounds, as by a meat diet. It is decreased by a vegetable diet.

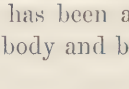
The organic sulphates normally make up about one-tenth of the total sulphates. The organic bases of these are such compounds as phenol (C_6H_5OH), cresol

($C_6H_4CH_3OH$), indoxyl (C_8H_6NOH), etc. These bases are formed by the putrefaction of albuminous substances; consequently, when such putrefaction is in progress in the body the organic sulphates increase in the urine. They may be formed in the intestine or absorbed from some other source. In the former case they are increased whenever there is a serious stoppage of the food, as in ileus or in peritonitis with atony of the intestine. In ordinary constipation there is no marked increase. In diseases which are accompanied by an internal formation of pus there is an increased amount of organic sulphates in the urine, and this fact may be used to judge whether the pus-forming stage has been reached. This is the case in fœtid bronchitis, carcinoma of the stomach or intestine, diphtheria, pyæmia, etc. If the formation is from putrefaction in the intestine it will be diminished by taking antiseptic remedies, like calomel, or those which, by their purgative action, remove the contents of the intestine before this putrefaction has occurred.

The compound of indol which is found in the urine goes by the name of indican. The indol,



formed by the putrefaction of albuminous substances, is oxidized after it has been absorbed from the intestine or elsewhere in the body and becomes indoxyl:—



This unites with potassium and sulphuric acid to form indican:—

$$\begin{array}{l} C_6H_4 - C - SO_4K \\ \quad | \quad \| \\ HN - CH \end{array}$$

Indican may be easily oxidized by chlorin or other oxidizing agents, and then forms indigo blue:—

$$\begin{array}{l} C_6H_4 - CO \quad CO - C_6H_4 \\ \quad | \quad | \quad | \quad | \\ HN - C = C - NH \end{array}$$

Putrefaction of nitrogenous compounds in the small intestine seems to be more productive of indican than when it goes on in the large intestine. Sometimes the indican is decomposed in the urine, the indigo being set free in the form of blue or red microscopic crystals. It is usually dissolved as a sulphate, however, until the indigo is formed by an oxidizing agent. It is normally present in large quantities in the urine of the horse, where, because of the long intestine, the residue from the food requires a considerable time to pass from the body.

Experiment 295. — Preparation of Potassium Phenyl Sulphate ($KC_6H_5SO_4$).—First prepare, if it is not at hand, potassium pyrosulphate by mixing 25 grammes of finely-powdered potassium sulphate with 15 grammes of concentrated sulphuric acid, then heating (best in a platinum dish). The heating should be done under a hood, to avoid the acid fumes. The heat should be gently applied at first, stirring until all the crystals have dissolved. When it ceases to bubble increase the heat to low redness. Allow it to cool, but before it solidifies it is best to carefully pour it upon a piece of clean

sheet iron. Powder finely the potassium pyrosulphate ($K_2S_2O_7$) thus obtained.

In a thin glass flask holding about a liter dissolve 15 grammes of potassium hydrate in 20 or 25 cubic centimeters of water, then add 25 grammes of crystallized phenol (carbolic acid, C_6H_5OH). When it has dissolved let it cool to 60° or 70° C., and, while stirring well, add gradually in small quantities 30 grammes of potassium pyrosulphate powdered as finely as possible. Keep it at a temperature of from 60° to 70° for from eight to ten hours, shaking often. Then add about 125 cubic centimeters of boiling 95-per-cent. alcohol, and filter while it is hot. This filtration is best performed in a hot-water funnel,—that is, one which is surrounded with a hot-water jacket. Otherwise the salt will crystallize out before the liquid has passed through the filter. As soon as the filtrate cools, the potassium phenyl sulphate crystallizes in pearly plates. It should be filtered out and recrystallized from a small quantity of boiling alcohol.

Experiment 296.—Test a solution of this organic sulphate with barium chlorid. There is no precipitate. Compare the result with that obtained from an inorganic sulphate, like magnesium sulphate, with barium chlorid.

Experiment 297.—Acidify a solution of an organic sulphate with hydrochloric acid, boil, and add barium chlorid. The acid has decomposed the sulphate; so that a precipitate of barium sulphate is now obtained.

Experiment 298.—Show that a mixture of the two classes of sulphates, as in urine, can be detected in this way. First acidify by acetic acid, then, after adding barium chlorid, let the test-tube stand at least half an hour in a beaker of boiling water. The inorganic sulphates are thus precipitated as barium sulphate, but not the or-

ganic. Filter, and test the filtrate with a drop of barium chlorid. If enough was added at first there will be no precipitate. If there is, more barium chlorid must be used, and the heating repeated. When the filtrate remains clear, acidify with hydrochloric acid and boil. The precipitate is from the decomposed organic sulphates united with the barium chlorid previously added.

Experiment 299.—Test urine for indican by adding to half a test-tubeful an equal volume of concentrated HCl. Then add a minute fragment of calcium hypochlorite ("chlorinated lime") and a few drops of chloroform and shake gently. Let the chloroform settle to the bottom. If indican is present in the urine it will be thus oxidized to indigo blue, and this colors the chloroform. A second piece of the hypochlorite may be added and the shaking repeated. An excess will destroy the blue color.

Albuminous Compounds of the Urine.

The principal albuminous substance occurring in the urine is serum-albumin. Besides this there may be found there serum-globulin, albumose, fibrin, and possibly peptones. The nucleoalbumins also are not uncommon, being often mistaken for mucin.

ALBUMINURIA.

Serum-albumin may find its way into the urine either from the kidneys (renal albuminuria) or from serous liquids,—like blood, pus, or lymph,—mixing with it at some point in the urinary tract below the kidneys. When it is due to degenerative changes in the kidney it is usually accompanied by epithelium from the tubules, often in the form of cylinders or casts. Changes in the composition

of the blood or in the blood-pressure may allow albumin to pass through the kidney. This is seen in anæmic conditions, after some poisons, and in some infectious diseases, the kidneys in any of these cases not being necessarily in a pathological state. Severe muscular labor may cause the temporary appearance of albumin. The quantity present varies greatly under different conditions, and is not necessarily a measure of the severity of the disease. Still comparative tests in the same case will indicate something of its progress.

The amount of albumin in the urine can be determined accurately by precipitating, drying, and weighing, but the process is a long one for clinical purposes. For a practical test, sufficient to show the variation in amount, Esbach's method can be used. This depends upon precipitating the albumin with a solution containing 1-per-cent. picric acid and 2-per-cent. citric acid. The operation is performed in a graduated test-tube, called an albuminometer, the height of the precipitate indicating its amount. Variations in temperature greatly effect the height of the precipitate; consequently in comparative determinations the conditions of temperature must be always the same. The results are most accurate when not more than 4 grammes of albumin are contained in a liter.

TESTS FOR ALBUMIN IN THE URINE.

If the urine is not clear it must be filtered before testing.

EXPERIMENT 300.—Heat the urine to boiling in a test-tube, then acidify with a few drops of concentrated nitric acid. If albumin is present, a white precipitate remains. The earthy phosphates precipitate on boiling, but are soluble in acids.

Experiment 301.—Pour half an inch of concentrated nitric acid into a small test-tube. Hold the tube in a slanting position and slowly pour upon the acid an equal volume of urine. If albumin is present a white cloud forms at the point of contact of the two liquids. If the amount is exceedingly small, it may not appear for half an hour. This is Heller's test. (If biliary pigments are present the ring may be colored. See test for these, Experiment 235.)

Experiment 302.—Acidify 2 or 3 cubic centimeters of potassium ferrocyanid solution with about 1 cubic centimeter of acetic acid, and fill the test-tube half full of urine. Albumin gives a white, cloudy precipitate.

Experiment 303.—Add to the urine in a test-tube about one-sixth of its volume of a saturated solution of sodium chlorid, acidify with acetic acid, and boil the upper part of the liquid, holding the tube by the bottom. Albumin gives a white precipitate, which shows plainly above the clear liquid in the lower part of the tube.

Each of these tests has some objections to it which must be recognized in interpreting the results. By the action of heat and nitric acid some of the albumin is decomposed; hence the first test is not as sensitive as some others. This decomposition is greatly increased if the urine is boiled after adding the acid. Besides albumin, there may be precipitated by this test uric acid from the urates in very concentrated normal urine, and also resinous matters after the administration of turpentine or the balsams. The resinous compounds are soluble in alcohol, which does not dissolve albumin. The uric-acid compounds are colored instead of being white, like albumin, and can be filtered out and tested.

The ring-test with nitric acid is very sensitive. It precipitates other substances than albumin,—such as the urates, mucin, and resinous substances. The urates do not form a ring at the plane of contact of the two liquids, but above it; and if the urine is previously diluted with two or three times its volume of water

they do not appear. The resinous matters dissolve in alcohol. The mucin precipitate forms a cloud in the upper part of the liquid where the acid is dilute. It dissolves in strong nitric acid.

Potassium ferrocyanid and acetic acid will detect very small quantities of albumin. Albumose is also precipitated if present. If the acid alone produces a cloudiness it is mucin or resinous compounds. These must be removed by filtration before adding the ferrocyanid.

In the sodium chlorid and acetic acid test the precipitate formed on boiling is acid albumin, which is insoluble in the salt solution. Resinous matters may be precipitated, but not mucus.

Experiment 304. Determination of Amount of Albumin in Urine by Esbach's Method.—The urine must not have a specific gravity above 1.008, otherwise it must be diluted. If it is not distinctly acid in reaction it must be made so by acetic acid. Fill the albuminometer with urine to the mark *U*. Add the reagent to the mark *R*. Close with a cork and mix gently, avoiding hard shaking, which introduces air bubbles into the precipitate and thereby prevents its settling. Let it stand at the temperature of the room (60° to 70° F.) for twenty-four hours. The height of the precipitate indicates the number of grammes of albumin per liter, or parts in a thousand.

GLOBULIN, ALBUMOSE, AND PEPTONES.

Globulin is found in the urine only with albumin. It passes into the urine in much the same manner, and has no especial diagnostic value. Albumose may be formed in urine by bacterial action from albumin. It may easily escape discovery, since it is not coagulated by heat. It is often the precursor of albumin, and, as such, a knowledge of its presence is important.

The results of the latest research have shown that much of what has been regarded as peptone in urine is one

of the albumoses which closely resembles it, and it is an open question whether peptones are ever found in this excretion. Nevertheless we may temporarily retain the name peptonuria for the condition, with the understanding that, as our knowledge becomes greater, it may have to be abandoned. The peptones or albumoses are not normally found in the blood, being converted in the intestinal mucous membrane into another form, probably into an albumin. When anything interferes with this conversion, or when they are otherwise introduced into the blood, they pass into the urine. Diseases of the intestine, like carcinoma or ulceration, may prevent conversion to albumin, giving rise to *enterogenic* peptonuria. Peptone and albumose are formed by the decomposition of albuminous substances by other means than by digestion; as, for example, by putrefaction. Diseases which are characterized by a formation of peptones are often accompanied by peptonuria. This is the so-called "pyogenic peptonuria." It is found when there is much formation of pus in a body-cavity, as in croupous pneumonia and with deep-seated abscesses. Before testing for its presence the other albuminous substances must be removed. After removing albumin by boiling the liquid acidified by acetic acid, albumose can be detected by its giving a precipitate upon saturation with sodium chlorid, which dissolves on heating and reappears on cooling.

The following tests can be used with urine containing a considerable peptone:—

Experiment 305.—Heat 50 cubic centimeters of urine to boiling; acidify if necessary with a few drops of acetic acid, filtering if it precipitates, and, while hot, add powdered ammonium sulphate to the filtrate as long as it dissolves and until there are some crystals in the bottom. Filter after

cooling. This leaves the peptone in solution. To insure complete precipitation of the other proteins the saturation with ammonium sulphate may have to be repeated. When this has been done and no further precipitate results, test portions of the filtrate with (1) tannic acid, (2) picric acid, and (3) potassio-mercuric iodid for peptone. Each should give a yellowish-white precipitate. The biuret test can be tried, but is not as sensitive as the others. With peptones, if no excess of copper sulphate is used, it should give a pink with no shade of blue.

FIBRINURIA.

Through hæmorrhage or exudation of serous fluids into the urinary passages the urine sometimes becomes mixed with fibrinogen, and this may form clots or semi-gelatinous masses. It may cover the bottom of the vessel or occasionally cause the whole mass to gelatinize. The fibrin can be filtered from the liquid through muslin and, after washing, can be tested (Experiments 67 and 70). It is very similar to the deposit of pus from fermenting urine. The pus, however, can be thinned with water. The fibrin is insoluble.

GLYCOSURIA.

Glucose is not normally found in large amounts in the urine, although traces are frequently—and perhaps always—present. More than a slight trace may be regarded as pathological if it continues for any length of time. A transitory form of glycosuria (alimentary glycosuria) is often caused by excessive quantities of carbohydrates, especially of sugar in the food. It may be produced by puncture of the fourth ventricle of the brain, by injuries of the pancreas, by a number of medicinal sub-

stances which act upon the vasomotor nerves of the liver, such as phloridzin, etc.

The urine is generally of a high specific gravity (1.030 to 1.050), having a light color and a whey-like odor. The daily volume may be increased to ten times the normal, the solids being likewise increased. When poured or shaken it retains the foam for a considerable time.

EXPERIMENT 306.—Test diabetic urine with

1. Trommer's test.
2. Fehling's test.
3. Böttger's test.
4. Bismuth subnitrate test.
5. Phenyl-hydrazin test.
6. Fermentation test.

Notice that the other urinary constituents may modify the results obtained with the solution of pure glucose.

For the detection of glucose in urine the tests already given may be employed (Experiments 25, 26, 27, 29, 30, and 31). No one of these, however, is an absolute proof of the presence of glucose. Other constituents of urine have a slight reducing power, and may respond to the tests with alkaline solutions of copper or bismuth, where the action is that of reduction, for example, uric acid and its salts; creatinin, mucin, and others occurring in smaller amounts have this power of reduction. The same is true of many medicines which pass into the urine. Trommer's and Fehling's tests are very sensitive under ordinary conditions, but they may fail in some decomposing urines, the ammonia which is present keeping the cuprous oxid in solution. Long boiling will expel the ammonia, and the test may then succeed. Large amounts of uric acid, creatinin, creatin, or albumin may act in the same manner, keeping the red oxid from precipitating.

In the test with the subnitrate of bismuth the salt is not so easily reduced by other compounds than glucose. Consequently

there is not so much danger of mistaking these for sugar. With a very large excess of the alkali this reduction may occur. This is said not to be the case with Nylander's modification of the test (Experiment 29). Albumin is, however, decomposed under such circumstances, giving a black precipitate. It must, therefore, be removed from the solution before the test is made. With this test very small quantities of sugar can be detected. Many medicinal substances pass into the urine and react with this test also.

The phenyl-hydrazin test is not affected by the reducing matters of the urine, but it gives a similar precipitate with milk-sugar. Pure phenyl-hydrazin must be used. If it is the hydrochlorid, the crystals should be white, not brown.

The fermentation test is not very sensitive. It may be interfered with by the presence of some drugs which stop the action of the yeast. If the urine is not acid, it should be made faintly so with tartaric acid. It can be used to distinguish between glucose and lactose. Barfoed's test (Experiment 27) may be employed for the same purpose.

EXPERIMENT 307.—Determine the quantity of sugar in diabetic urine, using Fehling's solution (Experiment 32). Dilute the urine with a measured volume of water if necessary, and use in the burette, as was done in the case of the pure glucose solution.

ACETONE, $(CH_3)_2CO$.

Normally acetone is present in the urine only in traces. Pathologically it occurs there in severe diabetes, in fevers, in inanition, and cachectic conditions, as well as in psychoses. In diabetes it often is a precursor of the more dangerous diacetic acid. It appears to be formed by the decomposition of albuminous compounds, and it can be produced in the urine by the use of a diet of such substances. It is a colorless liquid of a fruity odor, which boils at 56.5° C. and which can consequently be readily distilled from the urine. The examination of the urine should be made while it is fresh.

If a large quantity of acetone is present in urine the latter may be tested directly. For small amounts it is best to distil from about a liter one-fourth of its volume after slight acidifying with sulphuric acid. Place the distillate in a retort and distil from it about 30 cubic centimeters. This latter portion contains most of the acetone.

Experiment 308.—To a solution of acetone add a little sodium hydrate, then a solution of iodin in potassium iodid. Iodoform is produced as a yellowish powder having a characteristic odor. After a time it may form six-sided plates, which can be seen with a microscope. Notice also the odor. Alcohol gives the same result.

Experiment 309.—Prepare mercuric oxid by precipitating a little mercuric chlorid with sodium hydrate. Wash by decantation and filter and wash. Add this to some of the acetone solution, shake, and filter. The presence of acetone is shown by its dissolving the oxid. This can be proved by pouring a layer of ammonium sulphid solution on top of the filtrate in a narrow test-tube, when the mercury will be precipitated as a black ring between the two liquids.

Experiment 310.—To the liquid containing acetone add a drop of a freshly-prepared solution of sodium nitroprussid and make alkaline with sodium hydrate. A ruby-red color is produced. In a few minutes it changes to yellow. If it is acidified with acetic acid a carmin or purplish-red color appears if much acetone is present. On long standing (forty-eight hours) this changes to blue.

Diaceturia.

Diacetic or aceto-acetic acid ($CH_3COCH_2CO_2H$) never appears normally in the urine, but is found under the same pathological conditions as acetone. In the fevers

of childhood it is not so dangerous, but with adults it signals the approach of coma, of which it is, perhaps, the cause, through lowering the alkalinity of the blood. Diacetic acid is a colorless, strongly-acid liquid, soluble in water and ether. On heating it decomposes below 100° to acetone and carbon dioxid:—

$$CH_3COCH_2CO_2H = CH_3COCH_3 + CO_2.$$

With ferric chlorid it gives a violet-red solution, which disappears on standing twenty-four hours and more quickly upon boiling. This reaction can be used to detect diacetic acid in the urine. A number of other substances—like salicylic and carbolic acids, antipyrin, and the acetates—give a somewhat similar reddish color. These are stable at ordinary temperatures, and only that from the acetates is decomposed by boiling. The test should be made upon urine which has been comparatively freshly passed.

Experiment 311.—Test fresh urine for diacetic acid by adding, drop by drop, a solution of ferric chlorid as long as a precipitate forms. This is ferric phosphate, formed from the phosphates of the urine. Filter, and to the filtrate add a few drops more of ferric chlorid. The diacetic acid gives a violet-red color. Allow it to stand several hours, and notice that it fades and disappears.

Experiment 312.—If this violet-red color was obtained, heat another sample of the urine to boiling, and after cooling repeat the test. If the red color was caused by diacetic acid none will be obtained in this second test, since the acid will have been decomposed by boiling.

Lactosuria.

Milk-sugar may be found in the urine of women toward the end of pregnancy and a short time after child-

birth. Its presence indicates the absorption of the sugar from the fluid in the mammary gland. It may appear with the interruption of nursing or from stagnation of the milk in the gland. When the gland is well developed and lactose is found in the urine during the period of nursing it shows merely that the secretion of milk is abundant. The chemical reactions of lactose are very similar to those of glucose. The principal differences are that lactose ferments with yeast with difficulty or not at all, and that its power of reduction is less than that of glucose. Still, the distinction between the two as they occur in urine is a matter of some difficulty.

Experiment 313.—Try the fermentation test with compressed yeast, as in Experiment 31, upon urine containing glucose and that containing lactose, and notice that the former ferments, with the evolution of carbon dioxid, and the latter does not.

Experiment 314.—Try Barfoed's test (Experiment 27) upon the two kinds of urine, and notice that it responds to glucose, but not to lactose. In this test it must be borne in mind that the other reducing substances of normal urine—urates, creatinin, etc.—may cause a reduction of the copper salt.

Choluria.

In examining the urine for bile two classes of compounds are sought for: the biliary acids and the biliary pigments. The biliary acids do not normally occur in urine, except in small amounts. The pigments are more commonly found. In the freshly-passed urine usually only bilirubin is present, but by oxidation it may be generally of a yellowish- to greenish- brown color, and the changed to biliverdin, etc. Urine which contains bile is

sediment, if it contains epithelial cells, is often colored brown. Upon shaking the urine the foam is yellow or greenish.

A common cause for the appearance of the biliary constituents in the urine is the obstruction of the bile-duct. This may be either from some abnormal growth or merely from inflammation in the passages. The bile is then absorbed by the lymphatics and excreted through the kidneys. The same result may be produced by any abnormal condition of the liver which interferes with the free passage of the bile. A part of the bile may pass from the blood into the tissues, manifesting itself there by its characteristic color (icterus).

The biliary coloring matters may be formed in the liver, but they can also be produced by the decomposition of the hæmoglobin in the blood and the other tissues of the body, and may pass from here directly into the urine. In this case the urine would contain none of the biliary acids, since they do not appear to be formed outside the liver. A large amount of these acids with the pigments in the urine indicates that the bile comes from the liver (hepatogenous icterus). Some authors have described as a distinct form of icterus that where the biliary pigments are derived from the blood-coloring matters (hæmatogenous icterus). It seems, however, to be certain that the biliary acids may be absent from the urine even when it contains bile from the liver or gall-bladder.

Experiment 315.—Test biliary urine for the pigments by slowly adding urine to yellow,[1] concentrated nitric acid in a test-tube held in a slanting position. The acid remains in the bottom, and between the liquids are seen the colored rings, as in Experiment 236.

[1] The yellow acid can be made by allowing the colorless acid to stand for some time in a strong light.

EXPERIMENT 316.—If the urine contains much bilirubin, shake a large test-tubeful or more of urine with half an inch of chloroform; pour off the urine and let the chloroform evaporate on a watch-glass. The bilirubin is left in small, red prisms. It may be purified by dissolving in chloroform, filtering, and again evaporating. These crystals give the play of colors when moistened with nitric acid. They also dissolve in alkalies, and the solution becomes green on standing (biliverdin).

EXPERIMENT 317.—If the urine is dark colored from much urobilin or blood-coloring matters so that the colored rings do not show, test it with *Huppert's* test. Shake a test-tubeful of the urine with a small amount of milk of lime, then immediately pass into the liquid a stream of carbon dioxid to remove excess of lime. When it is neutral, filter and wash the precipitate, which contains the biliary pigments. Moisten the precipitate on the paper with a drop of moderately-strong, yellow nitric acid and observe the play of colors, from red to green.

EXPERIMENT 318.—In urine which is highly colored with other substances the bilirubin may be identified by Stokvis's test. To 20 or 30 cubic centimeters of urine in a test-tube add 5 or 10 cubic centimeters of a 20-per-cent. solution of zinc acetate. Wash the precipitated bilirubin upon a small filter; then dissolve it by the addition of a few drops of ammonia. The liquid which passes through the filter becomes, after standing, brownish green, and shows the spectrum of bilicyanin: an absorption-band between *C* and *D* and one between *D* and *E*. If much bile is present the liquid becomes blue upon slightly acidifying.

EXPERIMENT 319.—To the urine add a few drops of tincture of iodin. A green color results. If the iodin is flowed on to the top of the urine by slanting the tube a green ring is formed.

EXPERIMENT 320.—Test the biliary urine for biliary acids by dissolving in it a few crystals of cane-sugar, then dipping in it a strip of filter-paper. Dry the paper and place on it a drop of concentrated sulphuric acid. In a few seconds it becomes violet, best seen by holding it before a window. Too much sugar gives a brown color.

EXPERIMENT 321.—Instead of using concentrated acid make the test with dilute H_2SO_4, as in Experiment 219.

It is not advisable to depend upon Pettenkofer's test alone in the urine, as other substances may be present and give reactions similar to those of the bile-acids, although their spectra are different. The pure bile-acids may, in cases of doubt, be extracted by the following method:—

EXPERIMENT 322.—If the urine is highly colored or only a slight amount of bile-acids are present, it may be necessary to extract the latter before testing. Add to the urine lead acetate solution and a few drops of ammonia to make it slightly alkaline. Wash with water the precipitate, which contains the acids, then dry it. Extract it several times with warm alcohol, filtering hot. Make the filtrate alkaline with sodium carbonate, and evaporate to dryness on a water-bath. Dissolve the sodium salts of the bile-acids from the residue with hot, strong alcohol and filter. The bile-salts can be precipitated by adding ether to the cooled alcohol. They become crystalline on standing, or they can be tested for immediately in the alcoholic filtrate with Pettenkofer's or other tests.

HÆMOGLOBINURIA AND HÆMATURIA.

The hæmoglobin is found in the urine in two forms: first, dissolved, no corpuscles being present (hæmoglobinuria), and, second, in the corpuscles (hæmaturia).

The color of urine which contains blood is usually some shade of red, but may be dark brown or even greenish brown when the hæmoglobin has been changed to methæmoglobin. Very small quantities may not be detected by the eye. The liquid is often more or less cloudy from corpuscles and casts. There may be enough blood present to cause coagulation either in the urinary passages or after the urine is passed.

The free hæmoglobin is produced by the destruction of the corpuscles. This may be due to an injection of substances which dissolve the corpuscles, to the trans-

fusion of blood, to the action of some poisons and in certain infectious diseases, like typhus, also after severe burns. In this case the urine should be tested for hæmoglobin. If there is a sediment the microscope reveals no corpuscles.

Hæmaturia, where corpuscles are present, is more common. It is due to hæmorrhage in some part of the urinary tract. The corpuscles appear as a sediment and are usually not in rolls. They may be shriveled or swollen from standing in the urine. If the hæmorrhage is from the kidney, the blood is usually well mixed with the urine and of a reddish-brown color, the reaction being acid. Blood-casts may be present, and if they are it is a proof of a renal hæmorrhage. This may occur in Bright's disease, also with malignant renal growths or renal calculi.

If the hæmorrhage is from the bladder the urine is often alkaline, and clots of blood are common. It may be caused by vesical calculi, by cystitis or villous growths, and by carcinoma.

Experiment 323.—Add a very little blood to highly-colored normal urine, and notice that the bands of oxyhæmoglobin are visible through the spectroscope, although to the eye there may be no indication of its presence.

Experiment 324.—Convert the oxyhæmoglobin into hæmoglobin as in Experiment 198, and notice that the two bands change to one.

Experiment 325. — To urine containing a small amount of blood add enough sodium hydrate to make alkaline, and heat to boiling. The phosphates of the alkaline earths will be precipitated, and the precipitate will be colored reddish by the hæmatin from the decomposed hæmoglobin. If no blood were present the precipitated phosphates would be white. This test will detect very

small amounts of blood in urine. If the liquid is very dark colored, it may be necessary to filter and wash the precipitate before its color can be determined.

EXPERIMENT 326.—Examine microscopically the sediment from a urine after recent hæmorrhage. Observe the presence of red corpuscles and also the change in their form which takes place after standing.

MUCINURIA.

Both normal and pathological urine often contains a substance which, although similar to true mucin, yet differs from it in many respects. On account of this resemblance it is often called urinary mucin. The latest investigations indicate that it is a nucleoalbumin. In normal urine it appears after standing as a light, fleecy cloud in the middle of the liquid. Its origin is the mucous membrane,—principally that of the bladder, ureter, and vagina. In small amounts it has no special significance. In catarrhal inflammation of the bladder it is abundant. In cystitis and pyelitis it may give the urine a gelatinous appearance. Mucin is also increased in febrile conditions, as well as in nephritis.

Urinary mucin is precipitated from its solution by alcohol or dilute acetic acid without heating. It may be precipitated by very dilute mineral acids, but dissolves in excess. After precipitation by acids it is soluble in alkalies. Since nucleoalbumins, like the mucin of urine, are composed of an albuminous substance with a nuclein, they give most of the reactions of the albumins, such as those with potassium ferrocyanid, picric acid, the biuret test, etc. Care is necessary, therefore, to avoid confounding urinary mucin with small quantites of albumin. They can be differentiated by the fact that the mucin is precipi-

tated in the cold by acetic acid even after the urine has been diluted with water, while albumin is not.

Experiment 327.—Dilute normal urine with its own volume of water, acidify a small beakerful with acetic acid and allow to stand until the mucin has separated. Filter and wash with water.

Experiment 328.—Show that the mucin dissolves by adding a few drops of an alkali, like sodium hydrate, and that it is reprecipitated from this solution by acidifying again with acetic acid.

Experiment 329.—If urine containing much mucin can be obtained, apply the general tests for protein and albumin, and notice that it responds to many of them.

Lipuria, or Chyluria.

An abnormal condition of the urine—not uncommon among the inhabitants of the tropics, but more rare among those of cooler climates—is the presence of fat. Lipuria, or the appearance of fat in the urine, may be due to an abscess or fatty degeneration of the kidney; to an excessive amount of fat in the blood, as in pregnancy; or to conditions which produce fatty degeneration of other organs, as the liver, and in phosphorus poisoning, whereby the amount of fat in the blood is abnormally increased. The chyluria of the tropics is due to the action of a parasite, which causes a rupture of the lymph-vessels and allows the lymph to pass into the urinary passages. The urine is often milky, and, on standing, a creamy layer forms. It contains also the other constituents of the lymph, albuminous substances, etc. In cases of lipuria where only a small amount of fat is present it may appear in the form of drops upon the surface, or it may be present in microscopic globules, either free or in the casts or epithelial cells of the sediment. The globules can be perceived with the microscope and separated by ether.

Experiment 330.—Examine microscopically urine containing fat.

Experiment 331.—To half a test-tubeful of urine containing

fat add one-fifth its volume of ether away from the vicinity of a flame. Mix by shaking carefully. Allow to stand until the ethereal solution of fat rises to the top. Notice that the urine loses its milky appearance. Pour off the ether into an evaporating dish and let it evaporate without heating. Dip a strip of white paper in the residue, and notice that a greasy stain remains after drying.

URINARY SEDIMENTS.

Besides the soluble constituents of the urine, there are others which appear as an insoluble deposit upon the bottom of the containing vessel or floating in the liquid. They may be present in the freshly-passed urine or may appear after a time. The former are the more important to the physician, although some conclusions may be drawn from the latter as to the condition of the system.

For the collection of these sediments the best method is by the centrifugal machine, or centrifuge, this requiring so little time that the examination can be made before changes have occurred in any of the constituents. The centrifugal machine is essentially an apparatus where tubes or other vessels can be set in rapid rotation. These tubes swing from their upper end, and as the speed is increased assume a horizontal position. The solid constituents, being heavier than the liquid, are carried by the centrifugal force to the bottom of the tube. The tubes should contain from 15 to 20 cubic centimeters, and be rotated three to five minutes at a speed of at least 1500 revolutions per minute.

If a centrifugal apparatus is not at hand, the sediment is best collected by allowing the urine to stand in a conical glass vessel, containing 4 to 6 fluidounces, until it has settled. Then decant the supernatant liquid or

take, by means of a pipette, a sample of the sediment for testing.

Urinary sediments can be divided into two groups: the organized—or anatomical—and the unorganized—or chemical—sediments. Those of the first group are formed by vital processes, and of the latter by chemical force. Of the unorganized sediments some are soluble in acid and some in alkaline fluids. Their presence depends, therefore, upon the reaction of the urine. They fall naturally into two classes in accordance with their solubility, and may be farther subdivided according to their microscopic appearance. The table on the opposite page gives the most common varieties.

Before examining the sediment, test with litmus-paper the reaction of the urine in which it is found. Then place a drop of urine containing the sediment on a glass slide, cover with a cover-glass, and examine microscopically with a $^1/_2$- or $^2/_3$-inch objective. The microscopic examination should be made before the liquid evaporates and leaves on the slide the soluble compounds. A higher power may be used afterward if necessary, but generally the low power is preferable. Chemical reagents may be applied on the slide after removing the excess of urine by a piece of porous paper. Place one drop of the reagent on the slide by the side of the cover-glass. It will flow under the cover-glass, and its action can be observed with the microscope as it comes in contact with the different sediments. Care should be taken not to allow the reagents to touch the microscope-stage. If a low power is used without a cover-glass these tests may be made in a flat watch-crystal. Where large quantities of a reagent are employed, as in testing pus with an alkali, the ordinary chemical vessels are to be used.

Urinary Sediments.

Organized, or Anatomical.

- Pus.
- Mucus.
- Epithelium.
- Blood-corpuscles.
- Casts.
- Bacteria.
- Spermatozoa.

Unorganized, or Chemical.

In acid urine.

Crystalline.	*Non-crystalline.*
Uric acid.	Acid urates.
Calcium oxalate (envelope shaped).	Calcium oxalate (dumb-bell).
Triple phosphate (in very weakly acid urine).	Fat.
Acid calcium phosphate.	
Calcium sulphate.	
Tyrosin.	

In alkaline urine.

Crystalline.	*Non-crystalline.*
Triple phosphate.	Earthy phosphates.
Calcium oxalate.	Ammonium urates.
	Calcium carbonate.
	Calcium oxalate.

Urine containing pus is turbid when freshly passed, and gives the albumin reactions. When much pus is present it soon falls to the bottom as a thick sediment. Small quantities may remain suspended for a long time. In urine of an acid reaction the pus-corpuscles can be seen. They are circular and colorless, about twice the diameter of the red blood-corpuscles. They appear granular, but when brought in contact with acetic acid the granulation disappears and the nuclei, of which there are two or three, become visible. (Plate III, 13.) When the urine becomes alkaline, either by fermentation or by the addition of a fixed alkali, the corpuscles disappear and the mass becomes very sticky and gelatinous, so that it can be drawn out by a glass rod into long threads. The turbidity of the pustulous urine resembles that from urates or from the earthy phosphates. It does not disappear, however, like the former, by warming, nor, like the latter, upon the addition of acids.

The source of the pus in the urine may be anywhere in the urinary tract. When it is from the kidney the urine is apt to be acid in reaction, and round-celled epithelium or casts may be present. When it is from the bladder the urine is usually alkaline. It may be due to simple inflammation or to some deep-seated affection of the tissues.

Experiment 332.—Examine microscopically urine containing pus. Remove the excess of liquid around the cover-glass by means of a piece of filter-paper. Put a drop of acetic acid on the slide and let it run under the cover-glass. Notice the change in the appearance of the corpuscles.

Experiment 333.—Show that the turbidity does not disappear upon warming or upon acidifying.

Experiment 334.—Make Donné's test for pus by

allowing it to settle, then, after decanting off the urine, making it alkaline with sodium hydrate. The mass becomes extremely viscid, as is shown by stirring or pouring.

EXPERIMENT 335.—Show that the pus responds to the albumin reactions.

Mucus as a sediment is in the form of a slimy, viscid liquid, sometimes showing the mucous corpuscles. Its significance has been explained before. It can be made more visible by adding a little tincture of iodin, which colors it brownish. The addition of acetic acid to the urine precipitates mucin as a fibrous mass.

The epithelial cells, being continually thrown off from mucous surfaces, are normally present in small numbers in the urine. In such cases they are usually from the bladder and urethra, and, in women, from the vagina. A large increase, however, is indicative of a diseased condition of some part of the urinary system. The cells from different parts of the system are not all of the same shape. (Plate III, 14.) They may be considered as belonging to three classes: the squamous, or pavement-epithelium; the round celled; and the long, or spindle-celled, epithelium. The squamous epithelium is composed of large, flat, somewhat irregular cells with a distinct nucleus. They may be found singly or united, like the stones of a pavement. They occur chiefly in the outer layers of the mucous membrane of the vagina and bladder. The round-celled epithelium has smaller cells with a nucleus and nucleolus and are found especially in the tubules of the kidneys. They are also found in the deeper layers of the mucous membrane of other tissues, such as the bladder, urethra, and pelvis of the kidney. They are somewhat larger than the pus-corpuscles, and the nucleus can be seen without clearing by acetic acid. The long-celled epithelium is nar-

row and somewhat irregular, with a nucleus visible without staining. They are found in the outer layer of the membrane of the renal pelvis or in the deep layers of the bladder, ureters, and urethra.

Although the presence of a single kind of epithelial cells in the urine may give an indication of their origin, still their occurrence in different tissues often renders this a matter of doubt. The condition of the cells, however, may furnish information of the pathological changes which have taken place. If they appear disintegrated or contain fat-globules, their origin is from the locality of some degenerative process, often of a chronic nature.

Blood-corpuscles are not normal in urine. In freshly-voided urine they may retain their normal shape,—that of a biconcave disk. (Plate III, 13, *d*.) In acid urine, especially where the specific gravity is high, they shrivel after a time, the margins becoming irregular. In dilute urine and where the reaction is alkaline the corpuscles swell, and become biconvex or spherical. If there is much blood the liquid is reddish, but a slight amount may escape detection by the unaided eye. When it is present the albumin reactions can always be obtained.

By urinary cast is meant an irregularly-cylindrical mass, composed of various materials, which have been formed in the tubules of the kidney, and hence are of about the same size as the tubules. Opinions vary as to the cause of their formation, but most casts appear to be due to the coagulation of the serum which passes into the renal vessels owing to some pathological condition. The presence of anatomical elements—such as epithelium, pus, blood, and fat—or their decomposition products in the coagulated mass gives the different varieties of casts.

Epithelial casts are not very common. They consist

of cylindrical-shaped masses of round epithelial cells which are thrown off from the tubules by some pathological process. The cells may appear normal or they may be more or less decomposed and of a granular appearance, or they may contain minute fat-globules. The cells sometimes seem to compose the whole cast and sometimes to be scattered over its surface. (Plate III, 16.) When present, they indicate inflammation of the kidney. When the cells are degenerated, the indications are that the condition is chronic or has existed for some time.

Blood-casts consist of coagulated blood often containing so many red corpuscles that they are dark and non-transparent. They may be formed whenever hæmorrhage occurs in the urinary tubules, and are the best evidence of this. They are quite rare, and may be obscured under the microscope by the free blood-corpuscles.

Casts of pus are also very rare, but pus-corpuscles are not infrequently seen in other varieties of casts.

By the decomposition and metamorphosis of epithelium, blood- or pus- cells in casts, the so-called granular casts have their origin. They vary greatly in size, shape, color, and in fineness of granulation. (Plate III, 15.) The finely granular cannot be easily seen except with a high power of the microscope, although the coarsely granular may be observed with a low degree of magnification. They often contain unaltered epithelium, leucocytes, and fat-globules. Granular casts indicate degeneration or a long-continued pathological condition of the kidney.

Occasionally casts of fat-globules are observed. They result from farther metamorphosis of the granular casts. (Plate III, 18, *a*.)

In diseases of the kidney like interstitial suppurative nephritis, where bacteria are abundant, casts composed of

these organisms are often seen. They resemble granular casts, but are not destroyed by mineral acids and caustic alkalies, as are the granular casts. High powers of the microscope should be used in their examination.

Hyaline casts are almost transparent or at most show only a very fine granulation. On account of their great transparency they are extremely difficult to perceive. They may be colored yellow by adding a solution of iodin. In shape they are usually long and narrow. Besides these narrow hyaline casts, which probably are formed in the smaller tubules, there is sometimes found a broader variety. (Plate III, 17.) These have an indented edge and, in consequence of being more highly refractive, can be seen more easily than the narrow ones. They are called waxy casts. They often give the amyloid reaction,—a brown color with iodin, turning blue to violet upon acidifying with sulphuric acid. They are doubtless formed in the renal pyramids. The narrow casts dissolve readily in acetic acid, but the waxy casts remain in it for some time. Hyaline casts not infrequently have anatomical elements — blood- and pus- corpuscles, epithelium, etc. — clinging to the surface or included within the mass.

The origin of the hyaline casts seems to be due to the coagulable elements of the blood. It is doubtful if they are ever present in urine which has not been albuminous. Their presence, consequently, is indicative of the existence of albuminuria. They may be the best evidence of such a condition, as in interstitial nephritis, where the amount of albumin is small.

Whatever variety of cast may be present in urine, it shows, without any doubt, that there is a pathological condition of the kidney and that the accompanying albumin is of renal origin.

Besides these cylindrical casts there sometimes appear in the urine the so-called cylindroids. These are flat or ribbon-shaped, rather than cylindrical. They are usually about the diameter of casts, but longer, and resemble in their transparency and solubility the hyaline casts, their composition being probably the same. They are found in nephritis and congestion of the kidneys, also in cystitis. They do not seem to be characteristic of any pathological condition of the kidneys, but rather of some irritation of the lower urinary tract which has extended to the kidneys.

All casts are decomposed by bacterial action. The examination should, therefore, be made as soon as possible after the urine is passed and the casts have settled. This time may be shortened to five minutes by the use of the centrifuge. Without this it will be necessary to let the urine stand several hours or over night.

Experiment 336.—To examine urine for casts a few drops from the sediment obtained from standing in a conical glass or from the centrifuge is placed upon the microscope-slide; one with a shallow cell on top is best. Cover it with a cover-glass and remove liquid outside by filter-paper. Focus on the sediment, using a $^1/_5$-inch objective, then cut off nearly all light from below. When transparent or hyaline casts are sought for swing the mirror to one side and upward and throw the illumination upon the slide obliquely or use a small diaphragm. They will be more plainly visible by this means than by a strong illumination. After the casts have been detected their cylindrical shape can be shown by inclining the stage of the microscope so that they roll in the liquid.

Bacteria.

The freshly-voided normal urine contains no bacteria. They may be present, however, under abnormal conditions, and will soon appear in normal urine upon its standing exposed to the air. On account of the large amounts of organic matter dissolved in the urine, it furnishes a medium in which micro-organisms readily grow. This occurs even in the bladder if they are introduced from the outside, as, for example, by means of an unclean catheter. Urine containing bacteria is cloudy and is not cleared by filtration.

The non-pathogenic organisms are found in putrefying or decomposing urine. This is usually not acid and often is strongly ammoniacal. They may be found thus in the urine of cystitis where ammoniacal fermentation is excessive. Some of these are of large size and can be observed with a $^1/_5$-inch objective without staining. (Plate II, 8.) The pathogenic organisms are such as the pus-organisms, the diplococcus of gonorrhœa, and also the bacillus of tuberculosis and the organisms of infectious diseases. They can be examined and isolated by the common bacteriological methods.

Spermatozoa.

These may be found in the urine of males after coitus or pollution. They may be present in some diseases, like typhoid, and are constantly found in spermatorrhœa. By straining during defecation there may be a slight emission of semen, and consequently the spermatozoa be mixed with the urine. They are readily recognized by their characteristic shape under the microscope,—a flattened oval

head united with a long thread-like body and tail. (Plate III, 18, *c*.) They are most abundant in the first and last portions of the urine.

In freshly voided urine they may have some motion, but this soon ceases. Acids and alkalies, as well as pure water, stop it immediately. Spermatozoa resist putrefaction and the action of chemical reagents, even that of strong acids or alkalies.

Uric Acid and Urates.

The properties of these compounds have been given before. As a sediment, the free acid and its salts differ from all others in being colored yellow to brown. They are not abnormal in urine unless they are present as solids when the urine is passed, or are deposited within a few hours, since normal urine throws down uric acid on fermentation. The precipitation of these compounds is largely effected by a concentration or an increase in the acidity of the urine. The normal or dibasic urates are readily soluble in water, and do not occur in sediments. When the acidity of the liquid is increased, either by fermentation or by the addition of an acid, half the base is taken from these salts, leaving the monobasic or acid urates, which are soluble with much more difficulty. If the acidity becomes still greater, all the base is removed, leaving the free acid, which is only very slightly soluble in water. Of course, a decrease in the volume of water would be accompanied by a corresponding increase in precipitated uric acid and its compounds. Hence a sediment of these may appear in the urine without signifying that an increased quantity has been formed in the body. Thus, they are common in fevers, when the urine is of small volume

and concentrated. Less uric acid is formed in the body with a vegetable diet than with one of meat.

Uric acid and urates as sediments occur mostly in acid urine and can be usually identified microscopically. (Plate II, 11.) The color is characteristic. The acid is always crystallized, commonly oval or diamond shaped, sometimes visible to the naked eye, often in clusters or rosettes. The urates are commonly salts of sodium, potassium, or ammonium. They may be amorphous when examined with high powers. The so-called "brick-dust" sediment is a mixture of the sodium and potassium urates. Sodium urate is also found in fan-shaped clusters or irregular groups of fine crystals, and sometimes in granules. (Plate II, 8.) Ammonium urate makes up the "thorn-apple" crystals: brown, spherical masses covered with curved spicules. (Plate II, 9.) The urates can be differentiated from other sediments by being soluble on gently warming the liquid, as well as in alkalies. The urates, as well as the free acid, give the murexid test (Experiment 281). Uric acid is especially important when found as a sediment, from its tendency to form calculi. The same is true, to a less extent, of the urates.

Calcium Oxalate.

This salt is most frequent in acid urine. It may exist in two forms: the crystalline, or "envelope shaped," and the "dumb-bell shaped." Its appearance under the microscope affords the best method of identification. (Plate II, 10.) The crystalline form consists of octahedral crystals. They are never large, often being smaller than a red blood-corpuscle. When sufficiently magnified, they have somewhat the appearance of the back of a square envelope, the crossed lines being formed by the angles of the crystal.

In the shape of the crystals they resemble some forms of triple phosphate, from which they can be distinguished by their insolubility in acetic acid and by their smaller size. The amorphous form of calcium oxalate is disk shaped, with a contraction on opposite sides, so that it somewhat resembles a dumb-bell. Calcium carbonate has much the same form, but dissolves in acids with effervescence. Calcium oxalate is insoluble in acetic, but soluble in hydrochloric acid. The dumb-bell form gives rise to calculi of the bladder.

Oxalic acid and its salts are found in many fruits and vegetables,—like tomatoes, celery, rhubarb, etc.,—and when these are eaten it appears as the calcium salt in the urine. It is also produced in the body from certain foods,—as from large quantities of nitrogenous foods or from the carbohydrates, where the oxidation is not complete. A small amount, then, may be normal, and if it is transitory is of no great consequence. If the excretion is continual it is due probably to some constitutional weakness.

Phosphates.

The phosphates of the alkalies, being readily soluble in water, do not appear as urinary sediments. The phosphates of calcium and magnesium are insoluble in water or alkalies, although they dissolve in acids. They, consequently, appear as sediments whenever the urine becomes alkaline, but are not found in acid urine unless the acid reaction is very faint. They can be distinguished from other urinary sediments by dissolving in acetic acid without effervescence.

Triple phosphate, NH_4MgPO_4, is a salt of phosphoric acid having two bases,—ammonium and magnesium. When

it is made by precipitating a phosphate by ammonia and magnesium sulphate the crystals are usually stellate or snow-flake formed. As it is made in the urine, however, they are more commonly in the form of rhombic prisms. The terminations of the prisms are commonly truncated; so that the crystals have a shape which approaches that of the end of a coffin, and this gives rise to the common appellation: "coffin-lid crystals." (Plate II, 8.) The angles may not be so truncated and the long axis of the crystal may be so much shortened that it assumes the form of an octahedron, like the calcium oxalate. Unlike the latter, it is soluble in acetic acid. Calcium phosphate in the urine is usually amorphous, and always colorless. It is formed when the urine becomes alkaline in the absence of ammonia. To the eye it resembles pus, but differs from it in its solubility in acids. In acid urine the acid phosphate, $CaHPO_4$, may crystallize in long prisms, usually in clusters. Tribasic calcium phosphate, $Ca_3(PO_4)_2$, is colorless and amorphous. (Plate II, 7.)

The presence of phosphates may be due to an excessive formation in the body, and they are then usually accompanied by systemic disturbances. Alkalinity of the urine causes their appearance when there is no excess. This may be from the food or medicine, from an increase in the alkalinity of the blood, or from fermentation. Excessive mental work is often accompanied by phosphatic sediments. Their long-continued presence may excite fear of the formation of calculi. Their temporary appearance is a matter of no grave significance. In urine which has stood for a time after its passage they are the most common of the sediments.

Experiment 337.—Drop ammonia into normal urine until it is slightly turbid, and after it has settled examine

the sediment with the microscope. It is a mixture of the amorphous calcium phosphate and crystalline triple phosphate. To obtain a larger amount of the latter add to the urine a little magnesium sulphate before it is made alkaline.

EXPERIMENT 338.—Precipitate sodium phosphate with magnesium sulphate after making alkaline by ammonia. Notice the difference in the shape of these crystals under the microscope and those formed in the urine. Try the solubility of both forms in acetic acid.

EXPERIMENT 339.—Make normal urine alkaline with sodium hydrate and examine the precipitated calcium and magnesium phosphates with the microscope. Try their solubility.

CALCIUM SULPHATE.

This does not often occur as a sediment. It may be found in acid urines as long prisms united in clusters. (Plate II, 12, *a*.)

EXPERIMENT 340.—Prepare crystals of calcium sulphate by precipitating a rather dilute solution of calcium chlorid with a few drops of sulphuric acid. Dissolve the precipitate in boiling water, filtering hot if all does not dissolve. It will reprecipitate upon cooling. Examine with the microscope.

CALCIUM CARBONATE.

This compound is often found in alkaline urine with calcium phosphate. It appears as a sandy powder which, when examined microscopically, is seen to consist of spherical bodies formed of concentric layers or to have the dumb-bell shape of calcium oxalate. (Plate II, 9.)

It dissolves readily in acetic or other acids, with the evolution of carbon dioxid gas.

Tyrosin.

Tyrosin is not often found as a sediment because of its solubility in water, but it sometimes appears as such, though never in a normal condition of the system. It crystallizes in minute needle-shaped crystals, which are usually aggregated into clusters or sheaves. (Plate II, 12, *c*.) Its microscopic appearance is the best means of identifying it. The chemical tests have been given.

Tyrosin in the urine has the same source as in digestion: the decomposition of protein compounds. It is improbable that it comes from the intestine, but from other parts of the system. It is indicative of retrograde metamorphosis of the nitrogenous tissues. Thus, it is present in acute atrophy of the liver, in suppurative processes, and in phosphorus poisoning, which is accompanied by degeneration of the liver. Leucin is often found at the same time. (Plate II, 12, *b*.)

Fat.

The appearance and significance of fat in the urine (lipuria) has already been discussed.

SYSTEMATIC TESTING OF URINE.

In the systematic testing of urine the course is often varied, as the symptoms may point to the likelihood of the presence or absence of certain substances. The quantitative tests may be made use of or not according to circumstances. The following are the determinations

which are most important, with the tests which may be employed:—

1. Amount passed in twenty-four hours.
2. Color
3. Transparency
4. Odor

} Normal or abnormal. If the latter, what is the cause?

5. Chemical reaction.
   If alkaline, is it from NH_3 or fixed alkalies? (Experiment 270).
6. Specific gravity at 60° F. (15.5° C.).
7. Urea: quantity, percentage, and amount in twenty-four hours (Experiment 276 or 277).
8. Glucose.
   General test, Trommer's (Experiment 25).
   Confirmatory tests (Experiments 26, 29, 30, and 31).
   Quantitative test, Fehling's (Experiment 34).
9. Acetone.
   Experiments 308, 309, and 310.
10. Diacetic acid.
    Experiments 310 and 311.
11. Albumin.
    General test, heat and HNO_3 (Experiment 300).
    Confirmatory tests (Experiments 301, 302, and 303).
    Quantitative test, Esbach's (Experiment 304).
12. Blood.
    General test, spectroscope (Experiments 198, 199, and 200).
    Confirmatory tests, guaiacum test (Experiment 202; also Experiment 325).
    Corpuscles in the sediment.

13. Bile-pigments.
    General test, colors with yellow HNO_3 (Experiment 315).
    Confirmatory tests (317, 318, and 319).
14. Bile-acids.
    Experiments 321 and 322.
15. Peptone.
    Experiment 305.
16. Organic sulphates.
    Experiment 298.
17. Indican.
    Experiment 299.
18. Uric acid: amount.
    Experiment 285.
19. Total nitrogen: percentage and amount in twenty-four hours.
    Experiment 278.
20. Chlorin: amount.
    Experiment 290.
21. Phosphoric acid: amount.
    Experiment 294.
22. Identification of sediments, if present.

I. UNORGANIZED.

(*A*) *Crystalline.*
- Uric acid.
- Calcium oxalate.
- Calcium phosphate.
- Triple phosphate.
- Other rarer compounds.

(*B*) *Amorphous.*
- Urates.
- Phosphates, etc.

II. Organized.

Pus.
Mucus.
Blood-corpuscles.
Bacteria.
Spermatozoa.
Epithelium: kind and probable source.
Casts: kind and probable source.

The proof of the presence of any abnormal constituent should not be allowed to rest upon one test, but several should be tried.

URINARY CALCULI.

The constituents of calculi are the same as those of the chemical sediments, and the causes which give rise to the formation of the latter will also favor the production of calculi in the bladder. To these various names are applied, according to their size: sand, gravel, stone, and calculi, or concretions. They vary from the microscopic to aggregations as large as an orange. They are generally not composed of a single material, but have at the centre a nucleus, and this is surrounded by layers, often of two or more compounds in alternation. The nucleus may be a mass of foreign matter, or it may be a clot of blood or a particle of one of the sediments around which material, perhaps of a different kind, can be deposited. Uric acid concretions are the most common. They are brown in color, rough of surface, and brittle. The form of the crystals cannot be seen, but they give the murexid test. They dissolve in sodium or potassium hydrate, from which solutions the uric acid may be precipitated in the crystalline form by the addition of a mineral acid. Uric acid calculi are formed only in an acid urine.

The urates are often found mixed with the uric acid deposits or with those of calcium oxalate. The ammonium salt is the most abundant. They are generally small, grayish, and rather soft. They give the murexid test. They are deposited from acid urine, except the ammonium urate, which is formed in an alkaline solution.

Calcium oxalate concretions are commonly of large size and are very hard. The surface is rough and warty. They are called "mulberry calculi" from the resemblance of the surface to that of the fruit. The urine is generally acid, unless where the presence of the stone has produced cystitis. They are often dark in color from the blood which has been incorporated with them.

The phosphates can only be present in calculi when the urine is alkaline. They are generally rather soft and easily broken. Calcium phosphate has a chalky appearance. Triple phosphate, NH_4MgPO_4, is found with other substances. It is more commonly on the outside of the stone, being precipitated by the alkaline reaction produced by the presence of the concretion in the bladder. A mixture of the triple phosphate and calcium phosphate is fusible with the blow-pipe and is known as the "fusible calculus."

Calcium carbonate is not common, although found occasionally.

The analysis of calculi is made by the use of chemical methods. The stone should be broken or, better, if it is large enough, sawed through the middle. This shows the layers of which it is composed and the nucleus. If there appears to be any difference in the layers, they should be tested separately. Heat a piece upon platinum foil and notice whether it fuses and whether it is combustible or not. If it fuses it is an indication of a phosphate of cal-

cium and triple phosphate. If it is combustible it consists of organic compounds.

Blackening when ignited is evidence of organic matter, but if slight it may be merely mucus arising from irritation of the bladder, and not an essential part of the calculus. Ignition on the foil will divide the constituents into two classes, although both may be present.

Combustible, or Organic.	Incombustible, or Inorganic.
1. Uric acid.	1. Calcium phosphate.
2. Ammonium urate.	2. Calcium oxalate.
	3. Calcium carbonate.
	4. Triple phosphate.
	5. Urates of K, Na, and Ca.

If it is composed largely or entirely of organic matter try the murexid test (Experiment 281) for uric acid and urates. If inorganic compounds are present, powder a piece and treat in a test-tube with 2 or 3 cubic centimeters of dilute hydrochloric acid. Carbonates dissolve with effervescence of carbon dioxid gas, the others without. Warm, if necessary. Filter, if it does not give a clear solution. To one-fourth of the filtrate in a test-tube add sodium hydrate until it is alkaline, and test for ammonia by hanging in the tube a strip of moist red litmus-paper, being careful that it does not touch the side of the tube which is wet with the sodium hydrate. The tube can be allowed to stand corked over night or the ammonia-gas can be expelled from the liquid by boiling. If present it will turn the paper blue.

To the remainder of the solution in hydrochloric acid add ammonia until it is alkaline, acidify with acetic acid, and boil. If there is a precipitate, filter.

Precipitate is calcium oxalate. Test after washing and drying by heating to a bright-red heat on platinum foil. After cooling it should turn moist red litmus-paper blue.

To the filtrate add ammonium oxalate, boil, and, if there is a precipitate, filter while hot.

A white precipitate shows calcium, probably originally present as phosphate or carbonate.

The filtrate is to be tested for magnesium and phosphoric acid. For Mg make one-half alkaline with ammonia and if the liquid remains clear, add sodium phosphate. A fine, white crystalline precipitate with either reagent indicates Mg. For phosphoric acid make remainder acid with strong HNO_3 and add ammonium molybdate. A yellow precipitate appears.

Urates of K, Na, and Ca can be found by boiling the powdered calculus in water, filtering, and testing the filtrate by the murexid test. Or if it is evaporated to dryness and the residue is ignited on platinum the sodium and potassium will remain as carbonates, giving an alkaline reaction to litmus-paper.

THE METRIC SYSTEM.

In all work in modern chemistry the metric system of weights and measures is employed. The unit of length is the meter (39.37 inches); of weight is the gramme (or gram), which is the weight of 1 cubic centimeter of water at 4°; and, of capacity, the liter, which has the volume of 1 cubic decimeter.

Measures of Length.

10 millimeters	= 1	centimeter.
10 centimeters	= 1	decimeter.
10 decimeters	= 1	meter.
10 meters	= 1	decameter.
10 decameters	= 1	hectometer.
10 hectometers	= 1	kilometer.

Measures of Weight.

10 milligrammes	= 1	centigramme.
10 centigrammes	= 1	decigramme.
10 decigrammes	= 1	gramme.
10 grammes	= 1	decagramme.
10 decagrammes	= 1	hectogramme.
10 hectogrammes	= 1	kilogramme.

Measures of Volume.

10 milliliters	= 1	centiliter.
10 centiliters	= 1	deciliter.
10 deciliters	= 1	liter.
10 liters	= 1	decaliter.
10 decaliters	= 1	hectoliter.
10 hectoliters	= 1	kiloliter.

The following are especially to be remembered:—

One gramme is the weight of 1 cubic centimeter of water.

A liter contains 1000 cubic centimeters and a liter of water weighs, therefore, 1000 grammes.

The following are convenient in the conversion of the weights and measures of one system into another:—

1 meter = 39.37 inches.
1 foot = 0.304 meter.
1 liter = 61.03 cubic inches = 1.06 U. S. qts.
1 liter = 33.81 U. S. fluidounces.
1 gramme = 15.43 grains.
1 grain = 0.0648 gramme.
1 ounce (apoth.) = 31.1 grammes.
1 ounce (avoirdupois) = 28.35 grammes.
1 pound (apoth.) = 373.2 grammes.
1 pound (avoirdupois) = 453.6 grammes.

REAGENTS.

Fehling's Solution.—Make up and preserve in two parts: *A* and *B*.

(*A*) Dissolve 34.64 grammes of crystallized, non-effloresced copper sulphate ($CuSO_4$, $5H_2O$) in water and make up the volume to 500 cubic centimeters.

(*B*) Dissolve 173 grammes of pure, crystallized Rochelle salt (sodium and potassium tartrate) and 50 grammes of sodium hydrate in water, and bring the volume to 500 cubic centimeters.

Before using mix equal volumes of *A* and *B*.

Nylander's Reagent.—Dissolve in 100 cubic centimeters of water 2 grammes of subnitrate of bismuth, 4 grammes of Rochelle salt, and 10 grammes of NaOH.

Esbach's reagent contains, in a liter, 10 grammes of picric acid and 20 grammes of citric acid.

Millon's Reagent.—Dissolve 1 part of mercury in

2 parts of nitric acid (sp. gr., 1.42), first at ordinary temperature, then with the aid of heat. When it has dissolved add twice its volume of water, and after several hours decant the reagent from any sediment that may be present.

BARFOED'S REAGENT contains 0.5 per cent. of acetic acid and from 0.5 per cent. to 4 per cent. of cupric acetate in water.

GUNZBERG'S REAGENT FOR HCl.—Dissolve 2 grammes of phloroglucin and 1 gramme of vanillin in 100 cubic centimeters of alcohol.

BOAS'S REAGENT FOR HCl.—Dissolve 5 grammes of resorcin and 3 grammes of cane-sugar in 100 cubic centimeters of dilute alcohol.

Methyl-violet Congo red Tropæolin 00 Alizarin sodium sulphonate	A solution in water containing about 1 per cent. of the coloring matter.

Phenolphthalein, a 1-per-cent. alcoholic solution.

Dimethyl-amido-azobenzol, a 0.5-per-cent. alcoholic solution.

Iodin, a 1-per-cent. solution of potassium iodid, in water with a few crystals of iodin.

Of the common reagents, the following strengths may be conveniently used:—

Barium chlorid Ammonium hydrate Ammonium chlorid Tannic acid	10 per cent. in water.

Ammonium oxalate Ammonium molybdate Potassium ferrocyanid Potassium ferricyanid Lead acetate Sodium phosphate Ferric chlorid Copper sulphate Mercuric chlorid Sodium hydrate Silver nitrate	5 per cent. in water.
Picric acid Lime-water, or calcium hydrate	A saturated solution in water.

Sulphuric acid, 10 per cent.; pour 1 volume into 18 volumes of water.

Nitric acid, 10 per cent.; 1 volume of acid and 6 of water.

Hydrochloric acid, 5 per cent.; 1 volume of acid and 6 of water.

Acetic acid, 6 per cent.

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